THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXI MARCH, 1945 NUMBER 2

CHEMOTACTIC PROPERTIES OF BRUCELLA SUIS

A STUDY OF PHAGOCYTOSIS OF BRUCELLA IN VITRO BY NORMAL, NONIMMUNE HUMAN LEUKOCYTES *

J. W. DICKEY, JR., M.D., and WILEY D. FORBUS, M.D.
(From the Department of Pathology, Duke University School of Medicine, Durham, N. C.)

Although human brucellosis has been subjected to widespread and comprehensive clinical investigations, the details of the pathogenesis of the disease are little understood. Sharp,[1] in a recent summary of reports of investigations of this disease, makes this statement: "A more or less definite type of infectious granuloma is suggested, the most striking feature of which is a nodular lesion resembling the tubercle." From our own observations on the experimental disease [2-6] we are able to confirm Sharp's view in regard to the granulomatous nature of the basic pathological process in brucellosis, but we have not been impressed by any striking resemblance of the lesions to those of tuberculosis. On the other hand, from a review of the very few carefully studied fatal cases of brucellosis that are available, we have been impressed by a resemblance of the lesions to those of Hodgkin's disease.[7] That the latter resemblance may have some particular significance has been suggested by the observations recently reported from this laboratory [3,7-9] that brucella can be isolated from cases of Hodgkin's disease with impressive frequency. It is with the hope of learning more of the pathogenesis of brucellosis and thereby studying further the possible relationship between this disease and Hodgkin's disease that the following investigation of the influence of brucella over leukocytes from the peripheral blood was undertaken.

One of the first things that must be done in the study of the pathogenesis of any infectious disease obviously is to determine what reaction occurs immediately following the introduction of the organism concerned into the tissues. As a general proposition, we know that an immediate local accumulation of phagocytic cells is usually the first

* This work was supported in part by the Duke University Research Council and by a grant from the John and Mary R. Markle Foundation.
Received for publication, May 31, 1944.

thing that takes place. The general subject of phagocytosis in relation to infection and immunity has been of great interest since the work of Metchnikoff.[10] He thought that the polymorphonuclear neutrophils were the chief, if not the sole, agents responsible for resistance to bacterial invasion and that the failure of these elements to perform their phagocytic function would result in destruction of the host by the invader. Subsequently other workers [11-14] have adopted a somewhat different view, pointing to the fact that the circulating monocytes and especially the tissue macrophages appear to be the principal defenders of the body. Freedlander and Toomey,[14] for example, have shown that subcutaneously injected staphylococci are phagocytized by both types of cells, but are destroyed chiefly by macrophages. Likewise, Lucké, Strumia, Mudd, McCutcheon and Mudd [15] found that the degree of phagocytosis *in vitro* by the macrophages (including the monocytes) is approximately the same as that of the polymorphonuclear neutrophils. Furthermore, they and others have shown that while, in the experimental animal, the initial response to certain organisms (for example, the tubercle bacillus) is nearly the same for the cells of both types, in the later stages of development of the initial lesion, the monocytes and the macrophages always play the more important rôle.

If one takes into proper consideration these and other well known examples of the development of the initial cellular response to invading bacteria, it may be suspected that the characteristic features of brucellosis may depend to a great extent on the manner in which the phagocytic elements of the body react to brucella. A study of the phagocytosis of brucella *in vitro* by the white cells of the peripheral blood therefore appeared to us to be a logical point of departure for the study of this problem.

Experiments

Experimental Materials. In these experiments, Huddleson's strain 47 of *Brucella suis* from a 48-hour blood agar slant culture was employed; a nonhemolytic *Staphylococcus aureus* cultured in the same manner was chosen for comparative study. Whole human blood obtained by finger puncture with a capillary pipette was used as a source of leukocytes. For supravital studies, preparations were made with neutral red, pinacyanole and a combination of these two dyes (10 drops of neutral red and/or 10 drops of pinacyanole stock solution in 10 cc. of absolute alcohol) spread and dried in a film on the slide. Fixed preparations were stained with the following dyes: Wright's stain, hematoxylin and eosin, Kingsley's stain [16] and Giemsa's stain. Preparations for study were made according to slide-coverslip and hanging-drop methods as described below.

Experiment 1. Supravital Studies of Phagocytosis

A small amount of a saline suspension of live brucella was spread on a slide and upon this a small drop of blood was quickly placed; the preparation was then covered with a coverslip and sealed with a paraffin-vaseline mixture. Similar preparations were made using the staphylococcus. These preparations, on a warm stage in an incubator of 37° C., were observed for as long as 72 hours. Preparations supravitally stained with neutral red and/or pinacyanole also were made according to this technic. The leukocytes proved to be well differentiated in these preparations. The staphylococcus was readily identified both within and outside the cells, but brucella could not be identified except when the organisms were clumped; this was due to the small size of the organisms and to the confusion produced by particles in the plasma or by granules normally present in the cells. Smears of these same preparations were made by sliding the coverslip quickly off the slide; these were allowed to dry, after which the paraffin-vaseline was removed with xylene. These smears were stained according to a variety of methods as indicated above.

Experiment 2. Phagocytosis in Hanging-drop Preparations

Hanging-drop preparations for the study of phagocytosis of killed organisms were made in the following manner: A minute drop of distilled water suspension of organisms was placed in the center of a coverslip, allowed to dry and then gently heated for just a few seconds over a flame. This technic resulted in the production of a small ring of dead organisms on the coverslip. A drop of fresh whole blood was then allowed to clot over the organisms on the coverslip; following this, the coverslip was inverted over a hollow slide and sealed with paraffin-vaseline mixture. Preparations made in this manner were placed in the incubator and observed during periods varying from ½ to 72 hours. Because of difficulty in illuminating the central area of the hanging-drop of blood, observations on these preparations were restricted to the edge of the drop, quite beyond the ring formed by the concentration of organisms. This proved to be an unsatisfactory preparation and it was decided to apply Berman's method,[17] which consists of stripping the clotted drop away from the coverslip with filter paper and fixing and staining the remaining cells. Unfortunately, this usually produced a serious disturbance in the original arrangement of the cells. In further experiments it was determined that satisfactory preparations for study of the cells after incubation could be made by allowing the drop of blood to dry completely in the air, rinsing the dried blood with distilled water to dissolve the hemoglobin, and then drying again in the air. In

preparations of this type, the cells are killed quickly, are not disturbed in their arrangement and, when stained, are easily seen. Numerous stains were applied to these preparations, including Wright's, hematoxylin and eosin, and Kingsley's.[16] Brucella was not well stained by any of these; the staphylococcus stained very well with Kingsley's stain. The Giemsa staining method used by Wolbach in the study of *Rickettsia prowazeki*[18] was employed with excellent results.

Hanging-drop preparations for the study of phagocytosis of living organisms were made as just described except for omission of heating the dried suspension of organisms on the coverslip. (Drying for a short time does not kill all the organisms, as shown by cultures made from the coverslips.)

Control Preparations

Controls for the slide-coverslip method were made, using the same technic and vital stains but with no organisms; these were observed concurrently with the preparation of brucella and staphylococcus.

Controls for the hanging-drop method were made by touching sterile blood agar slants with the loop in the same manner as in making the suspension of organisms and duplicating each succeeding step.

Control of the phagocytic ability of the white blood cells of the blood employed under the experimental conditions was obtained through observation of the action of the whole blood cells toward the staphylococcus used for comparative study.

Experimental Observations

Living white blood cells were studied by the slide-coverslip method, both with and without supravital stains. In many of these preparations there were areas containing clumps of organisms and fluid, but no red cells. In a short time, these areas were invaded by polymorphonuclear neutrophils which rapidly cut swathes through the clumps of organisms, reaching out their pseudopodia and ingesting all of the organisms with which they came in contact. These pseudopodia conformed in shape exactly to the shape of the clumps of organisms and left no organisms behind when they withdrew. This process was observed to continue until the leukocytes were completely filled with organisms.

In the supravitally stained preparations, the staphylococci were seen clearly as unstained refractile bodies inside reddish stained vacuoles in the cytoplasm. The vacuoles were seen to increase in size, but the color became less intense as the cells gradually lost their vitality; after the cells died the vacuoles became colorless. The same type of vacuolization was seen in the preparation made with brucella following phagocytosis of these organisms, but the organisms could not be identified

inside the cells. In the control preparations without organisms this vacuolization began much later and was not nearly so massive. Neither staphylococcus nor brucella, dead or alive, regardless of their location within or outside the cells, was stained by the vital dyes. The organisms were not observed to grow in these preparations, perhaps because of exclusion of the air. No phagocytosis by cells other than the polymorphonuclear leukocytes was seen in these preparations. These cells were active for only a few hours; in contrast, in the unstained preparations they were active for a maximum of about 20 hours, after which they degenerated. In the unstained preparations, after about 20 hours the monocytes and lymphocytes exhibited active motion, which lasted as long as 60 to 70 hours. Even in the active state, these cells did not phagocytize either organisms or other cells. In the supravital preparations, death of these cells occurred within about the same time as that of the polymorphonuclear leukocytes.

In preparations supravitally stained with pinacyanole, phagocytosis by polymorphonuclear cells could be followed with ease. When cells are stained with this dye, the nuclei are dark purple and the cytoplasm light purple; mitochondria are also stained, as are some of the granules. The cells advance a clear layer of ectoplasm which contains no granules; from this many short finger-like processes extend. The ectoplasm first flows around an organism much as water flows along a surface and surrounds an object; next, the granular cytoplasm flows over the organism, which then moves with the rest of the streaming granules. Cells of other types move in a similar manner, but they were not seen to ingest the organisms.

In every hanging-drop preparation of either staphylococcus or brucella, dead or alive, observed from ½ hour incubation upward (with the exception of those in which the organisms were heated beyond a certain point), the neutrophilic polymorphonuclear cells in great numbers migrated to the ring of organisms on the coverslip (Fig. 1) and phagocytized numerous organisms (Fig. 2). The original distribution of the other white cells was not changed. In a few monocytes and eosinophils there were seen one or two organisms (these may have been superimposed), but certainly no marked phagocytosis by these cells was seen in any preparation. Control preparations made without organisms but with drops of distilled water which had been brought into contact with sterile blood agar showed no migration of cells.

When the organisms on the coverslips in the hanging-drop preparations were heated longer than a few seconds (not charred) they did not attract any polymorphonuclear leukocytes, were not phagocytized, and did not stain as they did in other preparations. Hanging-drop preparations made by drying without heating the organisms on the coverslip

showed, after incubation, that the organisms were still alive and had multiplied. In 12 hours, the polymorphonuclear leukocytes at the extreme periphery of the ring of organisms were completely filled with bacteria and some were surrounded by a small colony of organisms (Fig. 3). Comparing these preparations with the gently heated preparations it seemed that the organisms ingested by the polymorphonuclear cells had multiplied within these cells. (It was shown in the slide-coverslip preparations that the cells with ingested organisms are able to live as long as 20 hours.)

Comment

From the studies reported here, it appears that brucella offers little attraction *in vitro* for any of the normal circulating white blood cells except the neutrophilic polymorphonuclear leukocytes. This observation appears to be out of harmony with the general principle relating to phagocytosis as stated by Lucké and his associates,[15] namely, that "phagocytosis promoting properties of tropins apply similarly to macrophages and to polymorphonuclear leukocytes" and that "the mechanism of bacteriotropin action is the same for both kinds of cells." An explanation of this failure of brucella to attract normal monocytes is highly desirable in view of the fact that observations on the phagocytic activity of the tissue wandering cells—the tissue macrophages or clasmatocytes—in experimental brucellosis [3,5,19] have shown that these cells are commonly engorged with organisms. These latter observations are so impressive that it is suggested that brucella, at least, may propagate best in an intracellular environment. This apparent difference between the action of clasmatocytes and monocytes toward brucella may be a significant one, possibly indicating a fundamental difference between these cells. Such a distinction has been emphasized by Sabin and Doan [20] in their studies of the rôles played by the monocytes and the clasmatocytes in the development of the lesion in experimental tuberculosis. These workers suggest that phagocytosis is carried out chiefly by the clasmatocytes, whereas the monocytes form the tubercle in response to the irritating residue originating in the digestion of the tubercle bacilli by the clasmatocytes. (It is not illogical to postulate the operation of some such mechanism in the development of the brucella lesion and that this may lead to the development of cytological reactions such as those which characterize Hodgkin's disease.[21]) The problem obviously requires further study since in these preliminary experiments there are numerous factors as yet uncontrolled. The artificial conditions of the experiment doubtless were of some importance; this is suggested by the failure of the

staphylococci to attract the monocytes. Perhaps the properties of the particular strains of organisms used also may have been determining factors. Lastly, surely the concentration in the drop of blood of those immune bodies that usually influence the phagocytic activity of the leukocytes was material to the outcome of the experiments. A study of these and other factors concerned are problems for future investigation.

Summary

1. The behavior of white blood cells from the circulating blood of a normal nonimmune person toward *Brucella suis* (Huddleson's strain 47) was studied *in vitro* in slide-coverslip and hanging-drop preparations using fixed and supravital staining technics.

2. In the above described preparations, *Br. suis* provoked an immediate response on the part of the neutrophilic polymorphonuclear leukocytes and was phagocytized quickly by these cells; this response was not exhibited by any of the other leukocytes.

3. When ingested by a polymorphonuclear leukocyte, brucella was found to be surrounded by a vacuole. The ingested organism was not necessarily killed by the cell; in some instances it appeared to multiply within the cell body.

4. Brucella loses some of its staining properties and its chemotactic effects upon the polymorphonuclear leukocytes when it is heated beyond a certain point.

REFERENCES

1. Sharp, W. B. Pathology of undulant fever. *Arch. Path.*, 1934, **18**, 72–108.
2. Forbus, W. D., and Gunter, J. U. The pathogenicity of strains of brucella obtained from cases of Hodgkin's disease. *South. M. J.*, 1941, **34**, 376–389.
3. Forbus, W. D., Goddard, D. W., Margolis, G., Brown, I. W., Jr., and Kerby, G. P. Studies on Hodgkin's disease and its relation to infection by brucella. (Abstract.) *Am. J. Path.*, 1942, **18**, 745–748.
4. Brown, I. W., Jr., Forbus, W. D., and Kerby, G. P. The reaction of the reticulo-endothelial system in experimental and naturally acquired brucellosis of swine. *Am. J. Path.*, 1945, **21**, 205–231.
5. Margolis, G., Forbus, W. D., and Kerby, G. P. The reaction of the reticulo-endothelial system in experimental brucellosis of dogs. (Accepted for publication, *Am. J. Path.*)
6. Kerby, G. P., Brown, I. W., Jr., Margolis, G., and Forbus, W. D. Bacteriological observations on experimental brucellosis in dogs and swine. *Am. J. Path.*, 1943, **19**, 1009–1020.
7. Parsons, P. B., and Poston, M. A. The pathology of human brucellosis; report of four cases with one autopsy. *South. M. J.*, 1939, **32**, 7–13.
8. Parsons, P. B., Poston, M. A., and Wise, B. The pathology of human brucelliasis. (Abstract.) *Am. J. Path.*, 1939, **15**, 634–637.
9. Wise, N. B., and Poston, M. A. The coexistence of brucella infection and Hodgkin's disease. *J. A. M. A.*, 1940, **115**, 1976–1984.
10. Metchnikoff, E. Immunity in Infective Diseases. The University Press, Cambridge, Mass., 1905.

11. Buxton, B. H., and Torrey, J. C. Absorption from the peritoneal cavity. I. Absorption of particles in suspension. *J. M. Research*, 1906, **15**, 5–17.
12. Nakahara, W. The function of macrophages in local resistance to bacterial infections. *J. Exper. Med.*, 1925, **42**, 201–213.
13. Gay, F. P., and Morrison, L. F. Clasmatocytes and resistance to streptococcus infection. *J. Infect. Dis.*, 1923, **33**, 338–367.
14. Freedlander, S. O., and Toomey, J. A. The rôle of clasmatocytes and connective tissue cells in nonspecific local cutaneous immunity to staphylococcus. *J. Exper. Med.*, 1928, **47**, 663–675.
15. Lucké, B., Strumia, M., Mudd, S., McCutcheon, M., and Mudd, E. B. M. On the comparative phagocytic activity of macrophages and polymorphonuclear leucocytes. *J. Immunol.*, 1933, **24**, 455–491.
16. Kingsley, D. M. A new hematological stain. I. Constituents and methods of use. *Stain Technol.*, 1935, **10**, 127–133.
17. Berman, L. Observations on dry films of cultures of lymphoid tissue. *Arch. Path.*, 1942, **33**, 295–304.
18. Wolbach, S. B., Todd, J. L., and Palfrey, F. W. The Etiology and Pathology of Typhus. Harvard University Press, Cambridge, Mass., 1922.
19. Buddingh, G. J., and Womack, F. C. Observations on the infection of chick embryos with *B. tularense*, *Brucella* and *P. pestis*. (Abstract.) *Am. J. Path.*, 1941, **17**, 441.
20. Sabin, F. R., and Doan, C. A. The relation of monocytes and clasmatocytes to early infection in rabbits with bovine tubercle bacilli. *J. Exper. Med.*, 1927, **46**, 627–644.
21. Forbus, W. D. Reaction to Injury. Williams & Wilkins Co., Baltimore, 1943.

DESCRIPTION OF PLATE

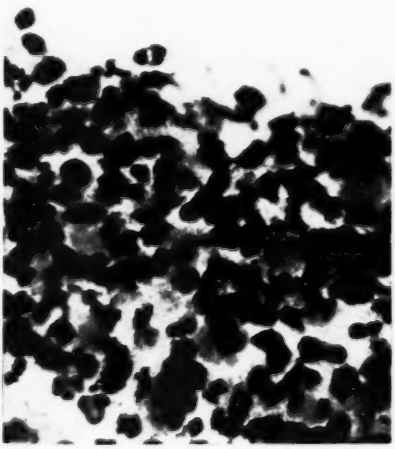
PLATE 32

FIG. 1. Hanging-drop preparation of brucella and nonimmune human whole blood, showing a ring consisting of polymorphonuclear neutrophils which have migrated to and ingested large numbers of organisms previously placed on the coverslip and killed by heating gently. Incubated 1 hour. Giemsa's stain. About × 40.

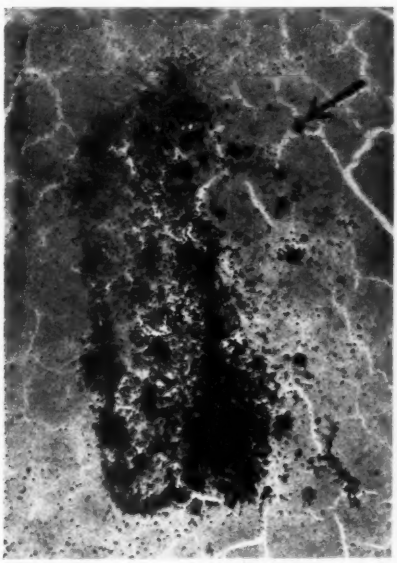
FIG. 2. A small segment of the periphery of the ring illustrated in Figure 1. The black nuclei of the polymorphonuclear neutrophils are shown, surrounded by cytoplasm packed with large numbers of organisms. The edge of the ring of cells is sharply defined; this outlines exactly the area containing organisms. About × 1100.

FIG. 3. A preparation similar to that of Figure 1, except that the organisms, brucella, were not heated and therefore many were not killed. Incubated 12 hours. The dark areas are masses of organisms which have grown during the incubation of the preparation. The arrow points to a colony of organisms surrounding an engorged polymorphonuclear neutrophil which has migrated from the ring carrying organisms with it. About × 40.

1



2



3

Dickey and Forbus

Chemotactic Properties of *Brucella suis*

THE REACTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN EXPERIMENTAL AND NATURALLY ACQUIRED BRUCELLOSIS OF SWINE *

IVAN W. BROWN, M.D., WILEY D. FORBUS, M.D., and G. P. KERBY, B.S.
(From the Department of Pathology, Duke University School of Medicine, Durham, N. C.)

In 1939, Parsons and Poston [1] reported that brucella may be obtained from an impressively high percentage of persons suffering from Hodgkin's disease. In this same report it was noted that it may be quite difficult to differentiate sharply between Hodgkin's disease and brucellosis either by clinical or by pathological-anatomical methods. The work of Parsons and Poston was followed by a clinical study of the relation between brucella infection and Hodgkin's disease by Wise and Poston,[2] whose work seemed to confirm the observations of Parsons and Poston. These investigations indicated a need for a critical study of the suggested etiological relation of brucella to Hodgkin's disease. Before studies of this type could be considered pertinent, however, it obviously was necessary to know something of the pathogenicity of the organisms that had been obtained from the cases of Hodgkin's disease. Forbus and Gunter [3] investigated this phase of the problem, and showed clearly that the strains of brucella recovered from the cases of Hodgkin's disease were capable of producing typical brucellosis in the guinea-pig; the disease produced in this animal, however, appeared to be distinctly less severe than that produced by organisms derived from naturally infected swine. All of these studies emphasized the need for a careful study of the reaction of the tissues to organisms of the brucella group.

It appeared most promising to approach the problems presented by such a study through an investigation of the reactions of the tissues of a variety of experimental animals to different strains of brucella. Accordingly, we have carried out a series of experiments involving the study of brucellosis in both natural and unnatural hosts; the animals employed were swine, dogs, monkeys, guinea-pigs, rabbits and chickens. A summary of our studies in swine and in dogs in combination with a brief note on the more comprehensive problem of the relation between Hodgkin's disease and brucella infection was published in the Proceedings of The American Association of Pathologists and Bacteriologists of 1942.[4] Subsequently, a detailed report of the bacteriological observations in our experiments on swine and dogs was published by Kerby, Brown, Margolis and Forbus.[5]

* This work was supported in part by the Duke University Research Council and by a grant from the John and Mary R. Markle Foundation.
Received for publication, May 31, 1944.

In this paper, our purpose is to record our observations on the reaction of the tissues of swine, both in brucellosis experimentally produced in our own laboratory, and in the naturally acquired disease as observed in a group of animals, tissues from which were obtained through the pathological laboratories of the United States Bureau of Animal Industry. The ultimate objective in these studies was to produce a disease which might be considered similar to, if not identical with, Hodgkin's disease in the human being.

Experimental Brucellosis in Swine

Almost without exception the experimental study of brucellosis in swine has been directed along bacteriological, immunological and epidemiological lines. In none of the studies that are available to us has particular attention been devoted to the pathological anatomy of the experimental infection. It is noteworthy that the few adequate descriptions of the experimentally produced lesions relate to observations made on the testis and related structures. Little or no attention seems to have been paid to the reticulo-endothelial system, in which it is generally known that the lesions in naturally acquired infections are commonly found. In the experiments recorded below, complete autopsies were always done, and special attention was directed to the reticulo-endothelial system.

EXPERIMENTS

Since our objectives could be obtained best through a study of prolonged chronic rather than rapidly terminating infection, inoculations were made with a sufficiently small number of organisms and at sufficiently infrequent intervals to assure infection that could be followed over a period of many months. A group of eight pigs, 8 weeks old, newly weaned by a sow from a farm on which no cases of brucellosis had ever occurred, was selected for the experiment. The sow and all of the pigs were studied bacteriologically and immunologically and found to be free of brucella infection. Two of the pigs (VII and VIII) were retained as normal controls for 207 and 205 days respectively and for 240 and 242 days respectively as infected controls. These animals were kept in separate quarters removed from all contact with the experimental group during the normal control period. The six experimental animals were kept in cages in the laboratory, the cages being just large enough to accommodate the animals at maturity. The control animals were kept under similar conditions. Both experimental and control animals were maintained on an adequate diet throughout the experiment.

Strains of *Brucella suis* designated respectively as "Brody" and "ABF 36" were selected for the inoculations. Strain "Brody" was obtained at autopsy from a case of Hodgkin's disease. Its virulence for guinea-pigs had been established.[3] Strain "ABF 36" was obtained from the spleen of a naturally infected hog (Fig. 14); this strain had been shown to be highly virulent for guinea-pigs.

Repeated inoculations of the six test animals were made at intervals of from 7 to 21 days. The inoculum was freshly prepared on each occasion from a 48-hour agar slant culture; it consisted of a saline bacterial suspension standardized by means of the photron-reflectometer. The number of organisms inoculated at one time (sometimes intraperitoneally, sometimes intravenously) varied, beginning with 70 million and increasing gradually to 30 billion. The maximum number of inoculations given to any animal was 32, and the minimum number, 6. Seven oral doses of 30 billion organisms each were administered to one of the normal controls, hog VII, beginning on the 205th day of the experiment, to provide infected controls. Repeated blood cultures were made during the experiment, and numerous cultures were made from the tissues at autopsy.[5] The agglutination titer of the blood for *Brucella abortus* 456 was determined at the time of each culture. The longest period of infection in any of the animals was 447 days, and the shortest period, 98 days. Table I summarizes the important experimental data. Sample protocols in summary are as follows:

Hog I. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog I, a male, 2 months old, weighed 15 lbs. It was fed on "Pig Grower," a commercial feed, supplemented with skimmed milk daily and cabbage every other day. Preliminary blood studies showed: opsonocytophagic index, 8.5; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 245 days after the first inoculation.

Hog I received 29 injections of brucella (Brody strain) beginning with 70 million and increasing rapidly to 30 billion organisms; the first 15 injections were intraperitoneal, the last 14 injections of 30 billion each were intravenous. Except for 3 or 4 injections all were given at intervals of about 1 week.

Temperatures taken every 3 days for the first 2 months ranged between 38.1° and 39.6° C. Opsonocytophagic index, initially 8.5, gradually rose to 46 on the day of death. Agglutination of brucella 456, initially negative, fluctuated widely from 1:2560 to 1:10240. Organisms were never recovered from the blood during the period of intraperitoneal inoculation. Ten colonies of brucella per cc. of blood were recovered 1 week after the first intravenous inoculation. Brucella was recovered 12 times from the blood thereafter.

During the whole course of the experiment this pig showed no symptoms or signs of disease, except for some lassitude for from 24 to 30 hours following intravenous inoculations.

Hog was killed by air embolism and immediately autopsied.

TABLE I

Experimental Brucellosis in Hogs: Summary of Experimental Data

Hog	Organism; Inoculations	Total days	Blood cultures and agg. titer	Autopsy cultures							Remarks
				Lymph nodes	Spleen	Kidneys	Liver	Lungs	Testicle	Peritoneum	
I	Brody; 15 i.p., then 14 i.v.	245/10*	21/12† 0/10,240‡	+	+	—	+	+	—	—	No clinical disease; positive blood culture at autopsy; no positive blood cultures during period of i.p. inoculation
II	Brody; 15 i.p., then 17 i.v.	436/117	24/9 0/10,240	—	—	—	—	o	—	o	No clinical disease
III	Brody; 14 i.p., then 17 i.v.	411/92	24/7 0/10,240	—	—	—	—	o	—	o	No clinical disease
IV	Brody; 14 i.p., then 18 i.v.	424/105	24/7 0/10,240	+	—	—	—	o	o	o	No clinical disease
V	Brody; 13 i.p., then 18 i.v.	447/128	23/9 0/10,240	—	—	—	—	o	—	o	No clinical disease; biopsy of spleen, liver, mesenteric node after 12 i.p. inoculations on 85th day; brucella recovered from spleen
VI	Brody; 14 i.p.	98/7	5/0 0/10,240	—	—	o	—	o	o	—	Hog died following weakness and anorexia of 33 days; no fever
VII Normal control, 207 days	ABF 36; 7 oral; infected control	240/129 Inf. control days	6/0 0/5,120	—	—	—	—	o	o	o	No clinical disease; cultures of blood always negative
VIII Normal control, 205 days	ABF 36; 6 i.v.; infected control	242/131 Inf. control days	6/2 0/10,240	+	—	—	—	o	+	o	Acute orchitis, right, 158 days before death; not completely healed at death

i.p. = intraperitoneal; i.v. = intravenous; — = negative; + = positive; o = no cultures.

* Numerator represents total duration of experiment and denominator the number of days between last inoculation and death.

† Numerator represents total number of cultures and denominator the number of positive cultures.

‡ Numerator represents initial agglutination titer for *Brucella abortus* (456); denominator the average titer following inoculations.

Hog V. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog V, a male, 2 months old, weighed 15 lbs. It was fed as was hog I. Preliminary blood studies showed: opsonocytophagic index, 3.5; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 447 days after the first inoculation.

Hog V received 31 injections of brucella (Brody strain), beginning with a dose of 70 million which was rapidly increased to 30 billion. The first 13 injections were intraperitoneal; the last 18 injections, of 30 billion organisms each, were given intravenously at weekly intervals.

The temperature was normal during the first 2 months, and not recorded thereafter. Opsonocytophagic index, initially 3.5, remained unchanged until intravenous inoculations were given; thereafter it varied widely, reaching 53 at the end of the experiment. Brucella 456 agglutination ranged between 1:1280 and 1:2560 during intraperitoneal inoculation; it rapidly rose to 1:10240 after intravenous inoculations were begun.

On the 85th day of the experiment, 8 days after the last of 12 intraperitoneal injections, specimens from the spleen, liver and mesenteric nodes were obtained for biopsy. Cultures of bile, peritoneal fluid and the above-mentioned tissues yielded brucella only from the spleen; paratyphoid bacillus was recovered from the nodes. The animal recovered from the operation and lived without clinical evidences of disease for 362 days.

The animal was killed by air embolism and an immediate autopsy was done.

Hog VI. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog VI, a male, 2 months of age, weighed 15 lbs. It was fed as was hog I. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative; blood culture, negative. The animal died 98 days after the first inoculation.

Hog VI received 14 weekly intraperitoneal inoculations of brucella (Brody strain) beginning with 70 million and increasing to 30 billion organisms. The last inoculation, on December 20, 1940, was made 7 days before death.

The temperature ranged between 38° and 39° C. The opsonocytophagic index, initially 7.0, rose to 14.5, but fluctuated as a rule between 4.5 and 11. Agglutination, initially negative, rose to 1:10240 1 week before death. Blood cultures were never positive.

Thirty-three days before death this hog developed weakness of the hind legs and had bouts of quivering which soon subsided. In spite of eating well for a while, it gradually became weaker and refused to stand and eat during its last few days. During this time no elevation of temperature was noted. The animal was found dead, having died 2 or 3 hours earlier; immediate autopsy was then done. Postmortem cultures were made from the spleen, liver, mesenteric nodes, retroperitoneal nodes, axillary nodes, heart's blood and peritoneal cavity and only postmortem invaders were found.

Hog VIII. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog VIII, a male, 2 months old, weighed 15 lbs. It was fed as was hog I. This animal was used as a normal control for 205 days; after this period it was inoculated with a control strain of brucella (ABF 36) which had been recovered from a naturally infected hog. Preliminary blood studies showed: after normal control period, blood culture

‡ Numerator represents initial agglutination titer for *Brucella abortus* (456); denominator the average titer following inoculations.

was negative; opsonocytophagic index, 17.5; agglutination for brucella 456, 1:160. The animal was killed 242 days after the first inoculation.

Hog VIII received 6 intravenous inoculations of brucella (strain ABF 36), each containing 30 billion organisms, from April 17, 1941 to August 16, 1941, the control infection period. The opsonocytophagic index rose from 17.5 to 31 but varied greatly. Brucella 456 agglutination, initially 1:160, rose to 1:10240 near the end of the period of infection. Brucella was recovered twice from the blood, the first time 1 week after injection.

One hundred and fifty-eight days before the animal was killed, it was noted that the right testis was swollen and tender. This continued until the testis reached a size one and one-half times that of the other on the 41st day of the disease; after this the swelling subsided. The involved testis then became smaller than its fellow and continued so until the animal was killed. No lymph nodes were palpable.

The hog was killed by air embolism; immediate autopsy followed.

Experimental Results

In Tables I, II and III are recorded the results of the experiments. The clinical and bacteriological observations need no descriptive statement, but the pathological-anatomical findings require detailed consideration.

Of the six animals inoculated with the Brody strain of brucella, only one (hog VI) appears to have died of the infection. The remaining five were killed. In these five animals no gross anatomical lesions were observed except in the lymph nodes and in the peritoneum.

Gross Lesions

The gross alterations of the *lymph nodes* consisted of a slight enlargement accompanied by what appeared to be extensive scarring (Fig. 1). In the scarred areas, particularly at the periphery of the nodes, there was pronounced hemorrhagic pigmentation. When sectioned, the surface was somewhat nodular, the little nodules presumably representing persistent lymphoid tissue; each nodule was isolated by a zone of what appeared to be hemorrhagic scar tissue. These changes were particularly prominent in hog I, in which the peripancreatic lymphoid masses were those most conspicuously altered. The lesions were least well developed in hog II. In this animal enlargement of the nodes was minimal, and hemorrhagic scarring was rarely observed.

On the *peritoneum* of each of the five sacrificed animals there were fibrous adhesions. In only one of this group, hog I, was there indication of testicular inflammation, a lesion that was so prominent in hog VIII, described below (Fig. 9). In hog 1, there were fibrous adhesions between the surface of the *tunica vaginalis*, accompanied by scarring of the testicular capsule. In the *testis* proper no changes were seen.

In hog VI, the one animal thought to have died of infection by the Brody strain of brucella, the *spleen* was somewhat enlarged and its

TABLE II

Experimental Brucellosis in Hogs: Gross Anatomical Findings

Hog	Spleen	Lymph nodes	Liver	Lungs	Testes	Miscellaneous lesions
I	Negative	Enlargement, scarring and hemorrhagic pigmentation of mesenteric, cervical, peripancreatic and retroperitoneal nodes	Negative	Negative	Negative	Fibrous adhesions between visceral and parietal laminae of tunica vaginalis testis; other viscera normal
II	Negative	Slight enlargement of cervical, mesenteric and peripancreatic nodes	Negative	Negative	Negative	Fibrous adhesions between loops of small and large intestine; other viscera normal
III	Negative	Slight enlargement of cervical, mesenteric and retroperitoneal nodes	Negative	Negative	Negative	Fibrous adhesions between small and large intestine and parietal peritoneum; other viscera normal
IV	Negative	Slight enlargement of cervical, mesenteric, peripancreatic and retroperitoneal nodes	Negative	Negative	Negative	Fibrous adhesions between peritoneal surfaces; other viscera normal
V	Negative	Slight enlargement of cervical, mesenteric and inguinal nodes	Negative	Negative	Negative	Fibrous peritoneal adhesions; other viscera normal
VI	Slightly enlarged, capsule roughened and dull	Enlarged, indurated cervical, mesenteric, peripancreatic, retroperitoneal and inguinal nodes	Focal thickening of capsule with fine, fibrinous and fibrous adhesions	Patchy congestion or pneumonia	Negative	Numerous fibrinous and fibrous adhesions between peritoneal surfaces, with small, encapsulated abscess-like pockets filled with necrotic material; entire peritoneum dull; other viscera normal
VII	Negative	Slight enlargement of mesenteric, cervical and inguinal nodes	Negative	Negative	Negative	Other viscera normal
VIII	Negative	Enlarged cervical, mesenteric, peripancreatic and inguinal nodes; large node on right spermatic cord, with focal necroses	Negative	Negative	Rt. testicle small, scarred, with partially calcified abscess-like areas of necrosis; similar changes in epididymis	Other viscera normal

capsule considerably thickened. There were incompletely organized adhesions attached to the surface of the spleen. In the *lymph nodes* there were changes quite like those in the other animals infected with the Brody strain. The surface of the *liver* was covered here and there by organizing fibrinous exudate. On the *peritoneal surface* of the intestine were scattered bead-like masses of exudate, which were of a somewhat caseous character (Fig. 8). These proved to be areas of granulomatous inflammation and they were distributed throughout the peritoneal cavity. With the exception of a few hemorrhages in the *heart* muscle, there were no significant changes in any of the other tissues.

Of the two animals inoculated with the ABF 36 strain of brucella, only one, hog VIII (inoculated intravenously), exhibited at autopsy unmistakable evidence of infection. In the other animal, hog VII (inoculated *per os*), nothing was found except a slight enlargement of the mesenteric, cervical and inguinal *lymph nodes*. In these nodes, however, minimal changes like those found in hog I were present. In hog VIII, which had received intravenous inoculations of the ABF 36 strain of brucella, a general enlargement of the lymph nodes was found. This was due chiefly to changes similar to those described in hog I; however, in addition to these changes an entirely different type of lesion was found in some of the nodes lying along the right spermatic cord. In these inguinal nodes there were foci of necrosis like those observed in the little caseous peritoneal nodules found in hog VI. In the right *testis* of hog VIII destructive lesions were found (Fig. 9). This organ was small, extensively scarred and dotted with little caseous foci; some of these had undergone calcification. A considerable amount of normal testicular tissue remained. The capsule of the testis was thickened by scar tissue, but was still intact. The *epididymis* was reduced in size, extensively scarred and, like the testis, spotted with foci of necrosis.

As a *normal control* for the gross alterations of the *lymph nodes* as just described we have used the submaxillary lymph nodes of ten normal hogs. The lymph nodes from the normal controls were approximately the same size as those of the experimental animals. Otherwise, however, there was a sharp difference. In the normal controls, the structure of the lymph node proved to be quite comparable to that of the normal nodes of man (Fig. 4). Comparison of these nodes with those of the experimental animals, especially hog I and hog VIII, left little doubt of the pathological state of the nodes of the experimental animals (Fig. 2). This interpretation was confirmed by the microscopical studies.

Microscopical Observations

The lesions described above require a detailed microscopical description since they have an important bearing upon the basic problem involved in our study. From the accompanying Table III it may be seen that our chief interest lies in the alterations of the lymph nodes and the development of the granulomatous reaction in the peritoneal cavity and in the testis and epididymis.

With the exception of hog VIII, in which orchitis and epididymitis occurred, the histological alteration of the *lymph nodes* in all of the experimental animals proved to be essentially the same. The characteristic lesion may be described as follows: The earliest change recognized was the massive accumulation of wandering cells of many varieties in the peripheral sinuses; most conspicuous were eosinophilic leukocytes, neutrophilic leukocytes, plasma cells, lymphocytes, and large, actively phagocytic, macrophages. In some of the lymph nodes there were great numbers of red blood cells. Occasionally, multinucleated, actively phagocytic, wandering cells were present. In the older lesions, the cellular masses that originally filled the sinuses, particularly those just beneath the capsule, were replaced by a remarkable growth of reticulo-endothelial cells having the appearance of a highly organized tissue (Figs. 2 and 3). In this reticulo-endothelial meshwork, the reticulum of which was demonstrated sharply by silver impregnation (Fig. 6), were many eosinophilic leukocytes, plasma cells, large mononuclear and multinucleated wandering cells and large macrophages (Fig. 5). In the far advanced lesions the leukocytes and other wandering cells were absent, leaving only the dense reticulo-endothelial structure, in which the reticulum fibers predominated. The disappearance of the wandering cells from the reticulo-endothelial areas sometimes was accompanied by the deposition of amorphous, hyaline material in the tissue spaces. Occasionally relatively large areas of necrosis and hyalinization were seen. In no place was anything resembling tubercle formation seen, and the reaction was not that which one would describe as epithelioid. The reticulo-endothelial reaction in these lymph nodes was, therefore, quite different from that which characterizes the response of the lymph nodes of the guinea-pig to brucella.[3] In some of the lymph nodes showing the sinus reticulo-endothelial hyperplasia the reaction extended to, and eventually completely replaced, the adjacent lymphoid tissue, thus converting the nodes into relatively homogeneous masses of newly formed reticulum fibers (Fig. 2). Red blood cells in the process of disintegration and some blood pigment could be seen in the earlier stages of development

TABLE III

Experimental Brucellosis in Hogs: Microscopic Findings

Hog	Lymph nodes	Spleen	Liver	Peritoneum	Miscellaneous lesions
I	Reticulo-endothelial proliferation of a granulomatous character with marked eosinophilia, hemorrhage and reticular scarring	Some thickening and scarring of capsule with a few macrophages	Some thickening of capsule	Fibrous adhesions	Occasional necrotizing granulomatous nodule in lung; organisms stained in retroperitoneal lymph nodes(?)
II	Dense reticular scarring in sinuses; eosinophilia	Fibrous adhesions over capsular surface	Capsular fibrosis	Fibrous adhesions	Organisms stained in peripancreatic nodes(?)
III	Dense reticular scarring obliterating architecture of nodes; eosinophilia	Negative	Capsular fibrosis	Fibrous adhesions	No other lesions
IV	Dense reticular scarring obliterating architecture of nodes; some active reticulo-endothelial proliferation	Negative	Capsular fibrosis	Fibrous adhesions	No other lesions
V	Dense reticular scarring obliterating architecture of nodes	Negative	Capsular fibrosis	Fibrous adhesions	Tissue obtained for biopsy after 12 i.p. injections showed active granulomatous peritonitis; no active lesions at autopsy
VI	Active reticulo-endothelial proliferation with mononuclear cells clogging sinuses; a granulomatous lymphadenitis	Granulomatous reaction involving capsule and splenic pulp just beneath capsule	Granulomatous reaction over capsule with mononuclear infiltration in periportal area, occasional small necrotic granulomatous foci in liver parenchyma	Active granulomatous peritonitis with focal necrotizing granulomata between intestinal loops	Mononuclear infiltration along fibrous tissue septa of epididymis; active granulomata in periadrenal tissue; organisms stained in peritoneum(?) and lymph nodes(?)
VII	Dense reticular scarring in cortical sinuses; eosinophilia and some active mononuclear proliferation	Negative	Negative	Negative	No other lesions
VIII	Dense reticular scarring obliterating much of the architecture of the nodes; active necrotizing granulomatous reaction in right spermatic node	Negative	Negative	Negative	Marked granulomatous involvement of testicle with necrosis and calcification; giant cell formation and scarring

of the lesion. Giant cells of the Langhans type rarely were seen, but multinucleated giant cells of other types were not rare in the earlier stages of development of the lesion (Figs. 5 and 7).

The histological picture just described was that characteristic of the lymph nodes in general. A significant variation of this picture was observed in the inguinal lymph nodes lying along the spermatic cord of hog VIII, in which there were also present an orchitis and epididymitis. In these lymph nodes, in addition to the changes already described, there were foci of necrosis, each focus being surrounded by a granulomatous inflammatory reaction; eosinophilic leukocytes and large multinucleated wandering cells were prominent in this reaction. The lesion in these lymph nodes was quite like that in the corresponding testis and epididymis, and in the peritoneum of hog VI (Fig. 8). Giant cells somewhat resembling the Langhans type occasionally were found near the necrotic foci.

In summary, the changes found in the lymph nodes in our infected animals can be described as those associated with the development of a granulomatous inflammatory reaction. This reaction was of such a character as to result eventually in complete replacement of the lymphoid tissue by a reticulo-endothelial scar (Figs. 2 and 3).

The histological reaction of the *peritoneum* was best illustrated by hogs V and VI. The most revealing tissues from hog V were obtained from the spleen by operation on the 85th day of the experiment, after 12 intraperitoneal inoculations of brucella had been made. The tissues from hog VI were obtained at autopsy on the 98th experimental day, after the administration of 14 intraperitoneal inoculations. (Death in this animal was related specifically to the brucella infection.) The inflammatory reaction of the peritoneum as shown in both of these animals was essentially granulomatous. The cellular exudate was made up predominantly of large mononuclear (sometimes multinucleated) wandering cells, polymorphonuclear leukocytes (eosinophils being especially prominent), plasma cells, lymphocytes, and large macrophages engorged with phagocytosed material. Necrosis of the exudate was found wherever it was abundant (Fig. 8). The lesion showed a profound tendency to heal, as indicated by the proliferation of fibroblasts and blood vessels. That healing eventually was complete was clear from the finding of nothing except fibrous adhesions in the peritoneum in hog V at the autopsy many months after the tissues for study were removed at operation. The discovery of peritoneal fibrous adhesions in all six of the experimental animals that received intraperitoneal inoculations appears to be a fair indication that granulomatous lesions such as those just noted must have developed in all of these

animals. Thus, disease due to brucella actually was established in all of these animals, even though the clinical and bacteriological studies of four of them left some doubt of established infection.

Certain other microscopical findings are of sufficient importance to be included here. One of these was the presence of focal granulomatous lesions in the *liver* of hog VI identical with those seen in typical brucellosis of the guinea-pig (Fig. 7). Accompanying these there was a marked portal round cell inflammatory reaction. The latter also was like that seen in brucellosis of the guinea-pig, and, furthermore, was like that described in hogs spontaneously infected with brucella.

Another lesion of interest was found in the *bones*. This consisted of a replacement of the marrow by edematous connective tissue and fat containing a variety of wandering cells. This, like the liver lesion, was most conspicuous in hog VI. Neither the hepatic lesions nor those in the bone marrow were characteristic of the animals as a group.

Lastly, in the *testis* and *epididymis* of hog VI, there was a fairly diffuse, chronic inflammatory reaction, but there was no lesion comparable to that in hog VIII now to be described.

The histological picture of the lesion in the *testis* and *epididymis* of hog VIII was that which frequently has been described in natural infections of swine and bovines by brucella. The characteristic lesion was an inflammatory reaction in which were mononuclear wandering cells and eosinophilic polymorphonuclear leukocytes in abundance, accompanied by a few neutrophilic leukocytes and a variety of small mononuclear wandering cells. The lesion seemed to develop somewhat along the following lines: The organisms appeared to have localized in the tissue surrounding tubules of the epididymis and the testis. This was followed by the development of the granulomatous reaction just outside the tubular structure and its extension through the lining of the tubules, finally to fill the lumen (Fig. 10). The exudate eventually became necrotic, and, at the same time, the epithelial lining of the tubules was destroyed. Necrosis was followed by deposition of calcium, after which the whole process subsided (Figs. 11 and 12). Complete healing resulted in the formation of a hyaline scar (Fig. 13). As the process healed a variety of giant cells, chiefly of the typical foreign body type, appeared in and about the partially calcified granulomatous lesion. Other types of giant cells were seen occasionally; some of these resembled the large multinucleated giant cells which characterize the Hodgkin's reaction but most of them were more like the Langhans giant cells. Nowhere did we observe typical tubercle formation; in fact, the reaction showed no striking resemblance to that produced by the tubercle bacillus, although the individual components of the reaction

were identical with those of tuberculous lesions. The result of the confluence of many lesions of the type just described was extensive destruction of both the testis and epididymis, reducing the whole to a contracted scar (Fig. 9). *In summary,* the testicular and epididymal lesion was essentially a granuloma which, though bearing a certain resemblance to the granulomatous lesions in a number of other diseases, was actually unlike any one of these; it probably is specific for brucella.

As may be seen in Table I, brucella was recovered at autopsy or at operation from the tissues of four of our eight animals. Nevertheless, in none of the tissues were we able to demonstrate the organisms by histological methods; this was so in spite of the fact that we routinely used for histological study those tissues from which the organisms had been obtained by culture.

Before discussing our observations on the reaction of the tissues of the hog to brucella in the experimentally produced disease, it will be useful to record our findings in a study of the naturally acquired disease in swine.

Naturally Acquired Brucellosis in Swine

Naturally acquired swine brucellosis has been studied clinically, immunologically, and epidemiologically by a great number of workers. The most comprehensive study of the problem is that of Thomsen.[6] Thomsen's work and that of more recent contributors, particularly Giltner [7] and Feldman and Olson,[8] have been summarized in Huddleson's monograph [9] and need not be reviewed here. It should be noted, however, that only a few of the students of this disease in the lower animals, particularly the hog, have devoted any attention to the extragenital lesions produced by brucella. Especially have the lymphoid tissues of the body, with the exception of the spleen, been neglected. Giltner, and Feldman and Olson have made good descriptions of lesions produced by brucella in the bodies of the vertebrae, and Christiansen and Thomsen [10] have made careful histological studies of the lesions in the genital organs in swine. Creech [11] seems to be the only student of the disease who has observed lesions in the lymph nodes.* It appears that naturally acquired brucellosis in swine is a relatively benign infection which only occasionally gives rise to lesions of any importance outside of the genital tract. The more important well known extragenital lesions are swelling of the joints, subcutaneous abscess-like lesions, abscess-like lesions in the bone, particularly the vertebral bodies, and caseous, terminally calcified, focal, granulomatous lesions in the spleen (Figs. 14 and 15). The lesions developing in the

* Creech describes very briefly a severe, caseating type of portal lymphadenitis in one hog.

uterus and responsible for abortion, those in the testis which result in sterility, and those in the bones appear from the descriptions to be essentially alike. They are granulomatous lesions with a tendency to necrosis and sometimes suppuration; complete healing occurs and results in scar formation and, occasionally, calcification.

Through the courtesy of Dr. C. L. Davis of the Denver Laboratory of the Federal Bureau of Animal Industry we have had an opportunity to study the lymph nodes and the spleen from four swine condemned by the meat inspection service because of the presence of lesions in the spleen that generally are thought by that service to be due to infection by brucella. From the lesions in the spleen in three of these animals, *Brucella suis* was obtained either by direct culture or by guinea-pig inoculation.

The lesion in the *spleen* which appears to be characteristic in these four animals is a granulomatous nodule which superficially resembles the tuberculous nodule of firm type commonly seen in man. Some of these nodules are caseous and some are quite hard; the latter consistency is the result of extensive calcification (Fig. 14). Microscopically the splenic nodules show at the periphery a mass of large mononuclear wandering cells, epithelioid cells, eosinophils and a variety of small mononuclear wandering cells. The center and the bulk of the lesion is formed by necrotic exudate, the cells of which are unidentifiable. Langhans giant cells are present, but giant cells of other types, presumably representing greatly enlarged and multinucleated wandering cells of the reticulo-endothelial system, are more common; these appear at the periphery of the lesion. In the old lesions, the necrotic inflammatory exudate is extensively calcified, and the lesion as a whole is surrounded by a zone of fibrous tissue (Fig. 15). The intervening tissues of the spleen are normal. We isolated *Br. suis* from an extensively calcified and encapsulated, but still active, lesion in one of our cases (this organism is that designated "ABF 36" and used in our experimental studies).

In only one of the four hogs with brucella lesions in the spleen were we able to study the lymph nodes, and these nodes showed no gross abnormality. In the peripheral sinuses and along the trabeculae in the interior of the node there was a granulomatous reaction with marked hyperplasia of the reticulo-endothelial elements and an excessive infiltration of eosinophilic leukocytes, an alteration of the lymphoid structure identical with that found in our experimentally infected animals.

From this brief description of our own observations, as well as from the observations of others, it is clear that *Br. suis* is capable of produc-

ing in the spleen a lesion which is essentially like that which it produces in the testis, the epididymis, and in the wall of the placenta of the pregnant sow.[6,10] It may be recalled at this point that in none of our experimental infections did we produce lesions of any type in the spleen.

Discussion

Before commenting on the changes that we have described in the lymph nodes of our inoculated animals, it is first necessary to determine whether or not infection by brucella actually was established in these animals. The details of this matter have been dealt with in a preceding paper,[5] but reference to Table I makes it possible to draw certain general conclusions. The following criteria have been used for determining active infection in our animals: (1) Repeatedly positive blood cultures not earlier than 1 week after inoculation; (2) high agglutination titers for brucella maintained throughout the experiment; (3) recovery of brucella from the tissues at autopsy as long as 131 days following the last inoculation; and (4) the presence of lesions known to be characteristic of brucella infection at autopsy or at exploratory operation. If judgment be based upon all four of these criteria separately or in combination, it seems fair to say that all of our inoculated animals had brucellosis even though only two of the eight presented clinical evidence of disease. In view of the difficulty in recovering brucella from animals known to be infected, and in view of the striking tendency of the lesions of brucellosis to heal, our failure to recover brucella from the tissues of all of our animals at autopsy cannot be considered a serious argument against the existence of infection in these animals.

In the course of our experiments we have found that the hog clears the blood of organisms within a period of from 1 to 3 weeks following inoculation of the blood stream with brucella; nevertheless, in animals in which this has occurred, the organisms can still be isolated from the tissues (lymph nodes) as long as 7 months after the inoculation.

Thus, it seems that brucella has a striking tendency to become isolated in the lymph nodes where it may remain and retain its pathogenic properties for a very long time. Under such conditions a mild but prolonged tissue reaction would be expected; indeed, alterations of the lymph nodes such as we have seen in our animals would be the natural outcome. Thus, it appears that we are entitled to attribute the lesions in the lymph nodes that are common to all of the experimental animals to the action of brucella.

One may ask why lesions in the lymph nodes of the experimental animals occur so regularly in the absence of changes in the spleen, the

bone marrow and other reticulo-endothelial tissues. Furthermore, why should the chronic, nonnecrotizing lesions in the lymph nodes in the experimental animals differ so greatly from the destructive lesions in the testis and the spleen (and occasionally in the lymph nodes) that occur in the naturally infected animals? We have no satisfactory answer to these questions unless it be that the hog is extraordinarily resistant to brucella infection and, therefore, usually suffers no destruction of its tissues, or that the necrotizing lesion is the only type commonly recognized by those who have most contact with these animals. The virulence of the organisms used in our experiments may likewise be an important factor. To this is to be added the significant fact that it is the lymph nodes that harbor the organisms for long periods of time. The literature dealing with swine brucellosis appears to be in harmony with this view of the matter.

Even though it appears permissible for us to interpret the lesions that we have described in the lymph nodes of our experimental animals as due to infection by brucella, this reaction in the lymph nodes cannot be considered peculiar to infection by this organism. The lesion is known to veterinary pathologists, who describe it as "chronic sclerosing lymphadenitis" and attribute it to a great variety of pathogenic agents. None of these agents appears to have been studied critically. Joest [12] accuses a variety of bacteria. From what we now know of brucella infections, not only in swine but in other animals, it may be that this chronic sclerosing lymphadenitis is a common expression of brucellosis in the lower animals.

A few comments may be made on the findings in our experimental animals as they may relate to the question of a possible relationship between brucella infection and human Hodgkin's disease. From the observations that we have made in the hog, it is not possible to say more than that brucella produces in this animal, as it does in other animals, a chronic, granulomatous form of reticulo-endothelial reaction, an inflammation, the essential features of which are those we recognize as belonging also to the basic reaction in human Hodgkin's disease. Thus far we have not been able to produce in swine by means of brucella inoculation a disease entity that can be considered similar to human Hodgkin's disease. That such an accomplishment is beyond possibility is, however, another matter. In fact, we know far too little about brucella infection, not only in swine and in other lower animals but also in man, to exclude such a possibility. From what we have seen in the hog, in the dog, in the rabbit, in the guinea-pig, in monkeys and, indeed, in man, it is clear that brucella has an extraordinary capacity to stimulate the proliferation and alteration of the reticulo-endothelial

system. Hodgkin's disease in man probably represents a reaction of the reticulo-endothelial tissues such as might well be produced by an agent with the pathogenic properties of brucella.

In conclusion, it is worth noting that there does occur in swine a peculiar granulomatous disease of unknown etiology that bears a certain resemblance to human Hodgkin's disease. Through the courtesy of Dr. C. L. Davis of the Federal Bureau of Animal Industry we have had an opportunity to study a group of swine affected with this disease. A report of these studies will be made in a subsequent communication, but it is worth noting here that we have attempted already to show a connection between swine brucellosis and this peculiar Hodgkin's-like swine disease by comparing the experimentally produced and naturally acquired brucellosis described in this paper, with the Hodgkin's-like disease in the group of animals just mentioned. At this writing it appears very difficult, if not impossible, to bring together as representing a single entity what we know to be brucellosis in swine and this Hodgkin's-like swine disease. Thus, the possibility of showing a relationship between swine brucellosis and swine "Hodgkin's disease" seems at this time to be surrounded by difficulties equally as great as those involved in the demonstration of a possible relationship between human brucellosis and human Hodgkin's disease.

Summary and Conclusion

1. A strain of *Brucella suis* isolated from a case of Hodgkin's disease and kept on artificial media for a long period has been shown to be pathogenic for the hog.

2. A strain of *Br. suis* freshly isolated from a naturally infected hog has been shown to be more highly pathogenic for the hog than the strain of *Br. suis* isolated from the case of Hodgkin's disease.

3. Hogs are highly resistant to *Br. suis* infection; they survive repeated large doses of the organisms given either intraperitoneally, intravenously, or by feeding.

4. The site of injury in the hog by *Br. suis* varies with the route of infection, but the reticulo-endothelial system, especially the lymph nodes, appears to be regularly affected.

5. Highly pathogenic *Br. suis* can be recovered from lymph nodes of hogs as long as 131 days after intravenous inoculation and in the absence of clinical disease.

6. The reticulo-endothelial cell with all its potentialities for morphological variation and proliferation is the chief reacting cell in swine brucellosis.

7. The lesions in the lymph nodes of the hog during the early stages

of *Br. suis* infection are characterized by: (a) marked proliferation of the reticulo-endothelial cells of the sinuses, the greatest number of cells developing into mature macrophages; (b) the presence of multinucleated giant cells; (c) marked infiltration of eosinophils; (d) occasional foci of necrosis followed by calcification.

8. The chronic lesions in the lymph nodes caused by *Br. suis* infection in hogs are characterized by a marked proliferation of the reticulo-endothelial cells resulting in replacement of the lymphoid structure by a dense reticular scar in the meshes of which a few eosinophils and mononuclear cells persist.

9. The healing of the necrotizing lesions of swine brucellosis, wherever they occur, is followed by reticulum-like scarring, occasionally with calcification.

10. It has not been possible in these experiments to produce in hogs by prolonged infection a disease entity that is comparable to human Hodgkin's disease; the basic reaction of the reticulo-endothelial tissues of the hog to brucella are, however, quite comparable to the basic reticulo-endothelial reaction that characterizes Hodgkin's disease in man.

11. There occurs in hogs a natural disease of unknown etiology, the lesions of which show a certain resemblance to those of human Hodgkin's disease. The nature of this disease and its possible relation to swine brucellosis requires further investigation.

REFERENCES

1. Parsons, P. B., and Poston, M. A. The pathology of human brucellosis; report of four cases with one autopsy. *South. M. J.*, 1939, **32**, 7–13.
2. Wise, N. B., and Poston, M. A. The coexistence of brucella infection and Hodgkin's disease. *J. A. M. A.*, 1940, **115**, 1976–1984.
3. Forbus, W. D., and Gunter, J. U. The pathogenicity of strains of brucella obtained from cases of Hodgkin's disease. *South. M. J.*, 1941, **34**, 376–389.
4. Forbus, W. D., Goddard, D. W., Margolis, G., Brown, I. W., Jr., and Kerby, G. P. Studies on Hodgkin's disease and its relation to infection by brucella. (Abstract.) *Am. J. Path.*, 1942, **18**, 745–748.
5. Kerby, G. P., Brown, I. W., Jr., Margolis, G., and Forbus, W. D. Bacteriological observations on experimental brucellosis in dogs and swine. *Am. J. Path.*, 1943, **19**, 1009–1020.
6. Thomsen, A. Brucella infection in swine. Studies from an epizootic in Denmark, 1929–1932. *Acta path. et microbiol. Scandinav.*, 1934, suppl. 21, p. 9.
7. Giltner, T. Report of the Chief of the United States Bureau of Animal Industry, 1930.
8. Feldman, W. H., and Olson, C., Jr. Spondylitis of swine associated with bacteria of the brucella group. *Arch. Path.*, 1933, **16**, 195–210.
9. Huddleson, I. F., Hardy, A. V., Debono, J. E., and Giltner, W. Brucellosis in Man and Animals. The Commonwealth Fund, New York, 1943, ed. 2.

10. Christiansen, M. J., and Thomsen, A. Histologische Untersuchungen über *Brucella suis*-Infektion bei Schweinen. *Acta path. et microbiol. Scandinav.*, 1934, suppl. 18, pp. 64–85.
11. Creech, G. T. Organic lesions in swine caused by *Brucella suis*. *J. Am. Vet. M. A.*, 1935, **86**, 211–216.
12. Joest, E. Handbuch der speziellen pathologischen Anatomie der Haustiere. Berlin, 1929.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 33

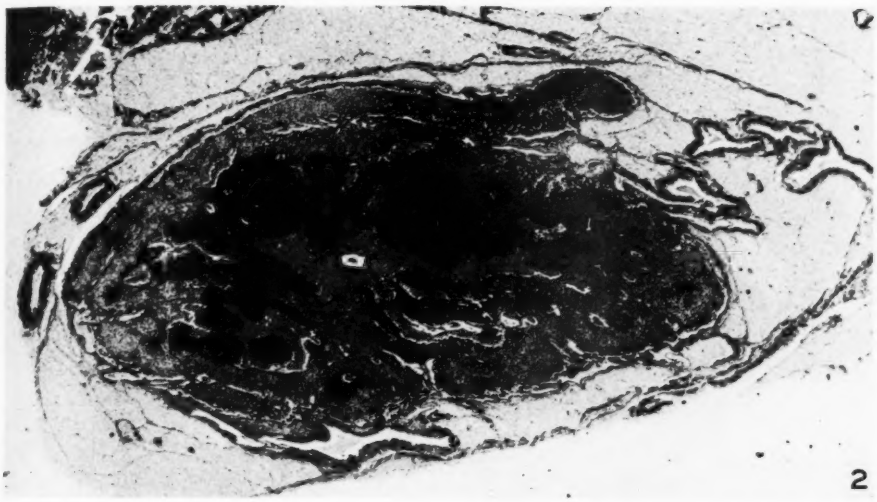
FIG. 1. Experimental brucellosis, hog I. Four lymph nodes, photographed at actual size, from a hog which had received within 245 days 29 inoculations of a strain (Brody) of brucella recovered from a case of Hodgkin's disease. The nodes are not significantly enlarged, but they show the scarring and hemorrhagic pigmentation characteristic of the nodes in all of the animals in this group. The two nodes at the left are seen in section and those at the right are unsectioned.

FIG. 2. Experimental brucellosis, hog II. Whole section of a small lymph node from an animal which had received within 436 days 32 inoculations of a strain (Brody) of brucella obtained from a case of Hodgkin's disease. The gray areas represent the reticulo-endothelial reaction, in the course of which the lymphoid structures are replaced. The black areas represent persistent lymphoid tissue. This may be compared with Figure 4, a photograph of a normal lymph node of a hog made at the same magnification. × 31.

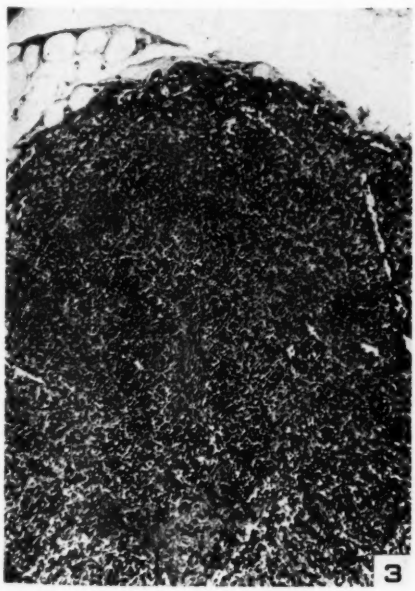
FIG. 3. Experimental brucellosis, hog I. Photomicrograph of a section of one of the lymph nodes shown in Figure 1. The tissue represented, similar to that in the gray areas of Figure 2, shows the granulomatous character of the reaction; no lymphoid tissue, as such, remains. This may be compared with Figure 4, a normal control, and Figure 5, a higher magnification of a similar reaction. × 130.

FIG. 4. Photomicrograph of a section of a normal lymph node from a normal control hog for comparison with the nodes of the experimentally infected hogs as illustrated in Figures 2 and 3.

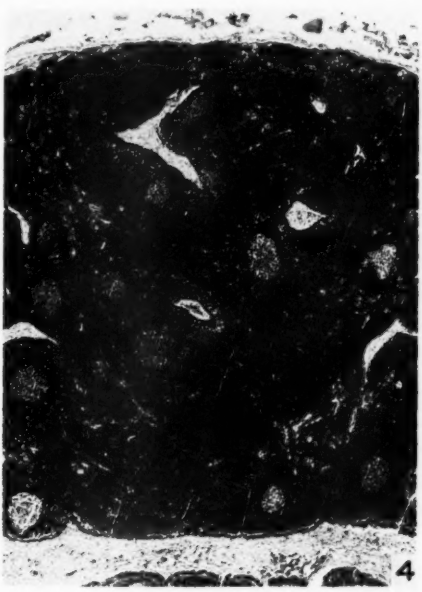
1



2



3



4

Brown, Forbus and Kerby

Reticulo-endothelium in Brucellosis

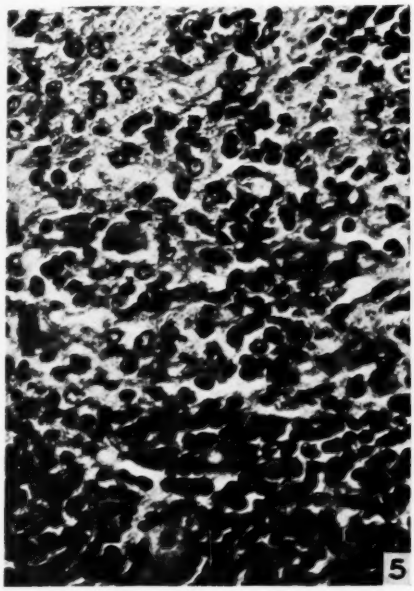
PLATE 34

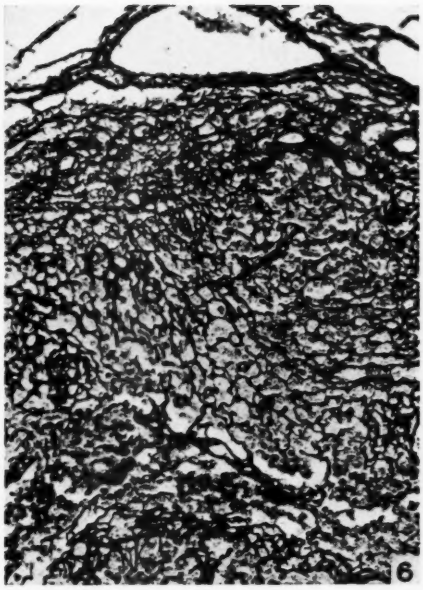
FIG. 5. Experimental brucellosis, hog II. Granulomatous reaction in a lymph node, like that shown in Figures 2 and 3 but at higher magnification. This animal had received 32 inoculations of the Brody strain of brucella within 426 days. × 395.

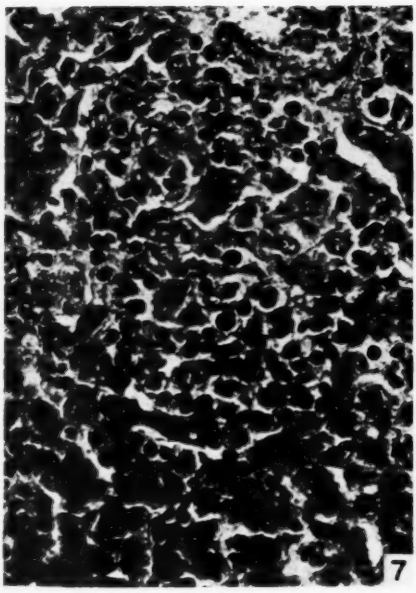
FIG. 6. Experimental brucellosis, hog II. Section of a lymph node like that in Figure 2, stained to demonstrate the reticulum of the reticulo-endothelial reaction which fills the peripheral sinus and extends to replace the cortical lymphoid tissue. × 245.

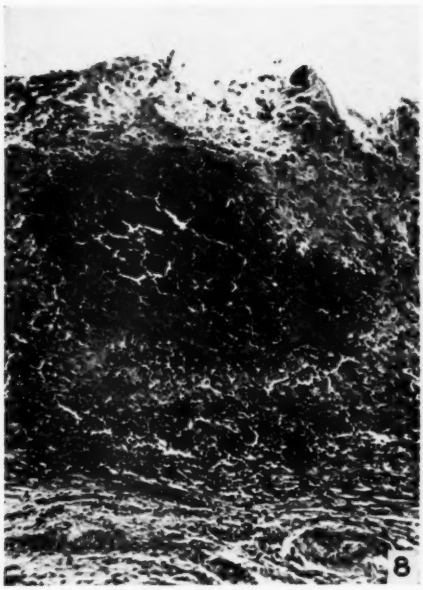
FIG. 7. Experimental brucellosis, hog VI. A focal granulomatous reaction in the liver near the portal area of the lobule from the only animal in the group that died of brucellosis. The cells of the reaction are eosinophilic leukocytes, large mononuclear wandering cells, lymphoid cells and multinucleated giant cells. This may be compared with Figure 8, a granulomatous reaction in the peritoneum of the same animal, dead on the 98th day; and with Figure 5, a granulomatous reaction in a lymph node of another infected animal that survived and was killed on the 426th day. × 395.

FIG. 8. Experimental brucellosis, hog VI. A necrotizing granulomatous lesion on the surface of the liver of an animal that died of brucellosis on the 98th day of infection by way of the peritoneum. This may be compared with Figures 5 and 7. × 130.









Brown, Forbus and Kerby

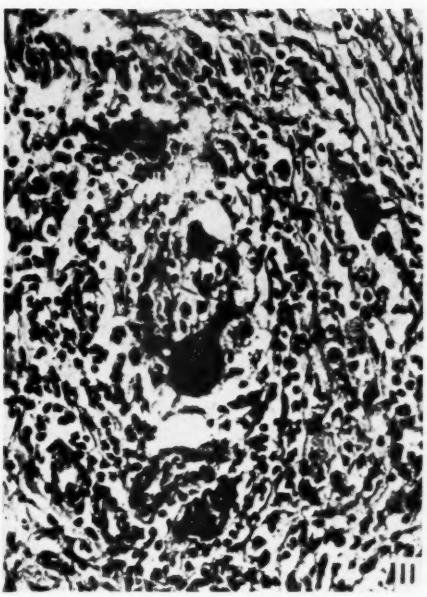
Reticulo-endothelium in Brucellosis

PLATE 35

FIG. 9. Experimental brucellosis, hog VIII. Healing granulomatous orchitis and epididymitis from an animal infected for 131 days with a strain (ABF 36) of brucella obtained from a partially calcified granulomatous lesion in the spleen of a naturally infected hog (Fig. 14). The normal left testis is shown for comparison. (The details of development of the characteristic lesions are shown in Figs. 10, 11, 12, and 13.) Two-thirds actual size.

FIG. 10. Experimental brucellosis, hog VIII. The earliest phase in the development of epididymitis and orchitis due to brucella. The lesion shown is a small granuloma which developed just beneath the epithelium of a tubule of the epididymis and subsequently destroyed the epithelium, spilling its substance, including the organisms, into the lumen. × 245.

FIG. 11. Experimental brucellosis, hog VIII. Granulomatous orchitis. This is a relatively early stage in the development of the lesion which is found in the supporting tissue of the testis between the seminiferous tubules. As in the epididymis, the lesion extends to and destroys the tubule; the granulomatous tissue finally takes the place of the tubular structure. × 245.



Brown, Forbus and Kerby Reticulo-endothelium in Brucellosis

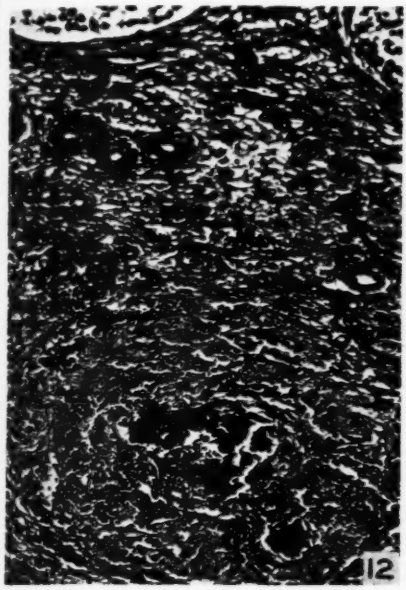
PLATE 36

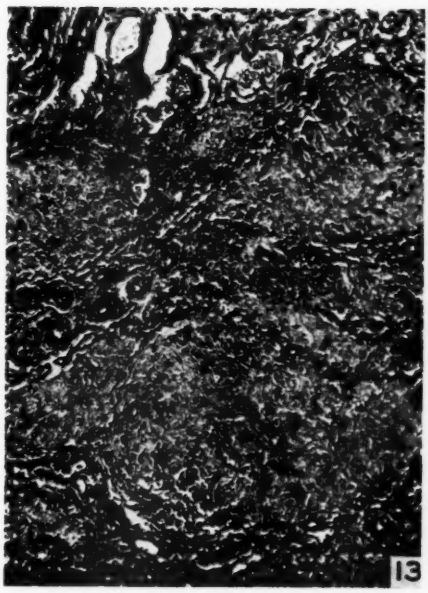
FIG. 12. Experimental brucellosis, hog VIII. A partially decalcified granulomatous lesion characteristic of the healing phase of orchitis due to brucella. × 130.

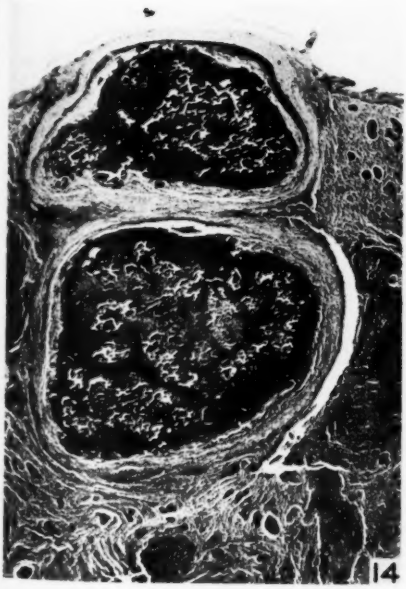
FIG. 13. Experimental brucellosis, hog VIII. Hyaline scars resulting from complete healing of lesions in the testis like that shown in Figures 11 and 12. × 245.

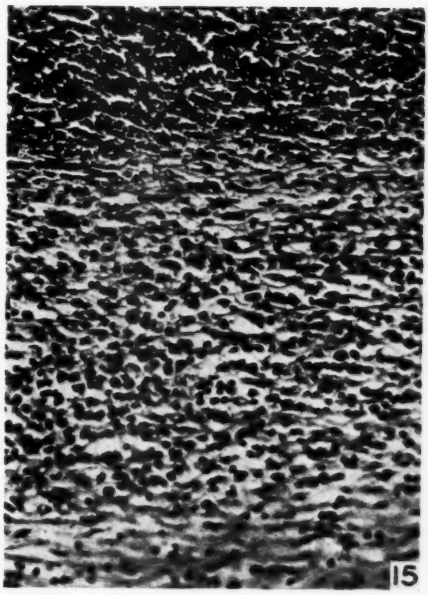
FIG. 14. Naturally acquired brucellosis, hog ABF 28. Two well encapsulated and partly calcified, but still active, granulomatous lesions in the spleen of a hog. The strain of brucella (ABF 36) used in the control infections in hogs VII and VIII was recovered from a lesion like this in another animal. × 27.

FIG. 15. Naturally acquired brucellosis, hog ABF 37. The tissue represented is the inner zone of the capsule of a lesion like that shown in Figure 14. The exudate is composed chiefly of eosinophilic leukocytes and mononuclear cells. In lesions of this type that are not so nearly healed, large mononuclear leukocytes and multinucleated giant cells form the inner border of this zone, next to the centrally situated necrotic mass of cells. The lesion is comparable to that shown in Figure 8. × 245.









Brown, Forbus and Kerby

Reticulo-endothelium in Brucellosis

THE RELATION OF HODGKIN'S DISEASE, LYMPHOSARCOMA AND RETICULUM CELL SARCOMA *

PETER A. HERBUT, M.D., FRANKLIN R. MILLER, M.D., and LOWELL A. ERF, M.D.
(From the Clinical Laboratories and the Division of Hematology, Department of Medicine, Charlotte Drake Cardeza Foundation, Jefferson Medical College, Philadelphia, Pa.)

Although medical literature is replete with publications on Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma, there are only sporadic reports on the relationship of these seemingly distinct entities. Gibbons,[1] in 1906, was one of the first to consider Hodgkin's disease as a variant of lymphosarcoma. Coley [2] shared this view and proposed that the name "Hodgkin's disease" be replaced with "lymphosarcomatosis." In 1913, Oliver [3] concluded that "all constitute a series of neoplastic processes of the lymphatic glands, which differ not so much qualitatively as quantitatively" and that "it is the predominate cell type which allows one to class the tumor as a lymphosarcoma, an endothelioma or Hodgkin's disease." Apparently Mallory [4] was of the same opinion for he considered lymphocytoma, lymphoma, lymphosarcoma, pseudoleukemia, lymphatic leukemia and Hodgkin's disease under the all inclusive term of lymphoblastoma.

A search of the more recent literature discloses only three pertinent case reports since 1928. One was that of lymphatic leukemia associated with reticulum cell sarcoma.[5] Another was a case of lymphoblastoma, Hodgkin's disease and tuberculosis [6] while a third was a case of Hodgkin's disease with sarcomatous features associated with lymphatic leukemia.[7] During this same period, however, general interpretations based upon large series of cases have been somewhat more numerous. Thus, concerning the relationship of Hodgkin's disease and lymphosarcoma, MacCarty [8] stated that they have "a common neoplastic cellular origin"; Levin [9] concluded that they are "phases of the same pathologic entity and the two may exist in the same patient"; Ginsburg [10] remarked that "they are merely variations of the same disease," and Cohn and Richter [11] were of the opinion that they are "genetically related." With reference to the relationship among lymphosarcoma, lymphatic leukemia, Hodgkin's disease and mycosis fungoides, Warthin [12] concluded that "transition forms exist between all of these groups and one type may be transformed into another." Commenting upon the same group of diseases, Sugarbaker and Craver [13] agreed that the borderlines between them "remain rather shadowy at present." Finally, the close relationship between the sarcomatous type of Hodgkin's disease and reticulum cell sarcoma has been clearly expressed by Callender.[14]

* Received for publication, April 22, 1944.

Because of the relative paucity of these reports, we shall consider in this paper a selected group of six cases that at one time were diagnosed as Hodgkin's disease and at another time as lymphosarcoma, and that at autopsy showed various combinations of Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma. In agreement with the above-mentioned observers, we believe that these processes are not only genetically related but that fundamentally they are merely phases of the same disease. This contention is supported both by the cases which follow and by the experimental work of Miller and Turner.[15] The latter will be fully elucidated in the discussion.

Report of Cases

Case 1

In May, 1939, a white woman, 45 years old, was admitted to another hospital complaining of weakness, generalized lymphadenopathy and enlarged spleen of 10 months' duration. Biopsy of a lymph node disclosed Hodgkin's disease. In May, 1941, an axillary node removed at the Jefferson Hospital showed a lymphosarcoma. Following x-ray therapy, her condition improved until September, 1943, when, in addition to the return of the previous signs and symptoms, she developed marked anemia and showed hypoplasia of the sternal bone marrow. From the third lymph node removed at this time, Hodgkin's disease was diagnosed. She died 3 months later.

The axillary node removed on May 29, 1941, measured 1.5 cm. in diameter. Microscopically, only a few follicles were still discernible and they were greatly enlarged, ill defined and in one or more areas blended imperceptibly with the cells of the adjoining parenchyma. Throughout the node most of the cells were large lymphocytes and the remainder were of the small variety (Fig. 1). Mitosis was occasionally seen.

A mass of cervical nodes removed on September 30, 1943, measured 2.5 by 1.5 cm. They were pinkish gray, moderately firm and contained several necrotic foci measuring as much as 3 mm. across. Histological sections disclosed a picture typical of Hodgkin's disease (Fig. 2). There were varying degrees of fibrosis, focal areas of necrosis, and a diffuse infiltration with large and small lymphocytes, plasma cells, a few polymorphonuclear leukocytes, scattered eosinophils and typical Sternberg-Reed cells. There were only a few poorly stained and ill defined reticulum cells in the background.

Autopsy performed on December 14, 1943, showed considerable emaciation and a "scaly" dermatitis. The lymph nodes in the left axilla measured as much as 2 cm. across while those of the mediastinum and along the abdominal aorta measured up to 5 cm. in diameter. They were matted together quite firmly and upon section disclosed pinkish

gray tissue with occasional foci of necrosis and hemorrhage. The liver weighed 1860 gm. and contained many circumscribed nodules measuring as much as 1.5 cm. in diameter. The spleen weighed 550 gm. but showed no tumor nodules. In the bone marrow of the lower thoracic and lumbar vertebrae there were irregular gray foci. Tumor nodules in the left pleura measured 1 to 2 mm. in diameter. The left pleural cavity contained 750 cc. of straw-colored fluid and the right pleural cavity contained 2000 cc. of similar fluid.

Microscopically, the lymph nodes exhibited three distinct pictures. All showed a complete disappearance of the normal architecture. In some there was a slight but uniform increase of reticulum and a diffuse infiltration with mature lymphocytes (Fig. 3). There were no other cells present. In other nodes there were scattered areas of necrosis, marked fibrosis and a cellular infiltration of lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and a few typical Sternberg-Reed cells (Fig. 4). The reticulum cells were ill defined and relatively inconspicuous. There were still other nodes that showed proliferation of reticulum cells to the exclusion of other cells and these varied considerably in shape and size (Fig. 5). Some were branched and their processes blended with the underlying reticulum fibrils, but most were detached and were irregularly shaped, rounded, or hexagonal. The cytoplasm of these cells was pink and abundant, and their nuclei were small or large, evenly stained or hyperchromatic, and occasionally they were in a state of mitosis. The only other cells present were a few scattered mature lymphocytes and occasional polymorphonuclear leukocytes. The portal canals showed large infiltrations of cells of the variety typical of Hodgkin's disease. Sections of the bone marrow showed similar cells but in addition there were large areas of necrosis and a diffuse early fibrosis. The spleen showed considerable hemosiderosis and some fibrosis but no other specific changes.

Summary. Thus the changes in the lymph nodes of this patient were those of lymphosarcoma in May, 1941, while they were those of Hodgkin's disease in September, 1943. Autopsy in December, 1943, showed lymphosarcoma, Hodgkin's disease and reticulum cell sarcoma.

Case 2

In May, 1940, a white woman, 38 years old, was admitted complaining of cervical lymphadenopathy and hematuria of 2 months' duration. A roentgenogram of the chest disclosed a mass in the anterior mediastinum. In spite of x-ray therapy she gradually developed increasing pallor, a persistent cough and loss of weight, and died in December, 1942.

A well encapsulated cervical lymph node measuring 1.5 cm. in diameter, removed on May 9, 1940, was composed of homogeneous gray

tissue with no gross areas of hemorrhage or necrosis. Microscopically, a portion of the node showed a diffuse infiltration with eosinophils; fewer lymphocytes, plasma cells and polymorphonuclear leukocytes; and clusters of typical Sternberg-Reed cells (Fig. 6). Faintly stained, drawn-out reticulum cells were sparse and fibrosis was slight to moderate. The remainder of the node was composed of circumscribed "tubercle-like" areas which consisted from the center outward of a small focus of eosinophils and polymorphonuclear leukocytes, a zone of necrosis, a rim of loosely arranged Sternberg-Reed cells, and finally a band of dense fibrous tissue.

On December 30, 1941, a mass of gray neoplastic tissue measuring 2.5 by 1 cm. was removed from the orbit. Histologically, practically all of the cells were of the large lymphocytic variety (Fig. 7). Mitotic figures were quite abundant. Occasionally, ill defined, poorly stained reticulum cells and a few thin-walled capillaries were seen in the background. There were rare polymorphonuclear leukocytes but no Sternberg-Reed cells were seen.

Necropsy performed on December 4, 1942, showed emaciation and protrusion of the left eye. The lymph nodes of the left axilla, the mediastinum and the abdomen were variably enlarged, reaching their maximum size in the epigastrium where they measured 14 by 12 by 8 cm. In the region of the thymus they formed a flat mass, 10 by 6 by 1.5 cm. Everywhere they were matted together, firm and uniformly gray. None showed necrosis or hemorrhage. The spleen weighed 250 gm. and contained circumscribed gray tumor nodules measuring as much as 1 cm. in diameter. Similar but larger tumor masses were present in the left lung. Some of these, however, showed central areas of necrosis. The remaining organs, including the liver and vertebrae, showed no pertinent abnormalities.

Microscopical sections of many of the nodes showed a pathological process similar to that found in the orbital mass described above (Fig. 8). Nearly all of the cells were of the large lymphocytic series. There were only a few scattered polymorphonuclear leukocytes and plasma cells and no Sternberg-Reed cells. In other areas, however, and in the lungs the cells consisted of various proportions of lymphocytes, eosinophils, polymorphonuclear leukocytes, plasma cells and occasional Sternberg-Reed cells. There were also foci of necrosis. Sections of the nodules in the spleen were similar to those from the cervical lymph node described above except that there were more areas of necrosis and fibrosis. Some sections of the lumbar vertebrae showed areas composed entirely of lymphocytes while others contained, in addition,

eosinophils and plasma cells. There was an extensive purulent bronchopneumonia.

Summary. Biopsy in May, 1940, showed Hodgkin's disease of a granulomatous type. Biopsy in December, 1941, disclosed a picture resembling lymphosarcoma. Autopsy in December, 1942, showed a combination of the two processes.

Case 3

A white boy, 17 years of age, was admitted in March, 1942, complaining of a mass in the neck. This had first appeared 8 years previously following an attack of measles but had grown rapidly 1 month prior to admission. In spite of x-ray therapy he gradually developed weakness, pallor, loss of weight, an enlarged liver, pains in the back and legs and anemia, and died in September, 1943. Repeated sternal punctures showed an increasing replacement of the bone marrow with lymphoid tissue.

A group of "enlarged," loosely bound, discrete cervical nodes was removed on April 10, 1942. These nodes were uniformly pinkish gray and showed no necrosis or hemorrhage. Microscopically, the normal architecture was completely replaced by a diffuse and dense infiltration of mature lymphocytes (Fig. 9). Throughout the sections there were occasional thick-walled and thin-walled capillaries, surrounding which there was some increase in collagen. Attached to the latter were scattered, faintly stained, ill defined reticulum cells. There were no other cells present.

Necropsy was performed on September 27, 1943. The lymph nodes of the submaxillary regions, the supraclavicular fossae and the left infraclavicular fossa were enlarged to 1.5 cm. in diameter. The mediastinal and all abdominal nodes were much enlarged and matted together, reaching their greatest size along the abdominal aorta where they measured 16 by 6 by 4 cm. On section they were uniformly gray and showed no areas of necrosis or hemorrhage. The spleen weighed 810 gm.; the liver, 1790 gm.; and the kidneys, 120 gm. each. Each of these organs contained gray nodules of varying sizes.

Microscopical sections showed a complete disappearance of the normal architecture. There was some increase of reticulum cells. Many of these were elongated and their borders were either branched or otherwise irregular; others were somewhat rounded. All contained an abundant amount of cytoplasm and had round, regularly stained nuclei. Lymphocytes were greatly reduced in numbers but both the large and small variety were present. The remaining cells consisted of polymorphonuclear leukocytes, plasma cells and many Sternberg-Reed cells containing from 1 to 20 piled-up nuclei (Fig. 10). The outlines of

these giant cells were usually round but occasionally their borders were branched, assuming the appearance of reticulum cells. In addition to cells of the above variety, there were scattered areas of early necrosis. Sections of the spleen, liver and bone marrow showed essentially the same microscopical appearance.

Summary. Biopsy of a cervical lymph node showed a lymphosarcoma of the small cell variety. Autopsy 1½ years later disclosed typical Hodgkin's disease.

Case 4

A white man, 57 years old, was admitted to the hospital in September, 1943, complaining of general lymphadenopathy, cough, weakness, dyspnea, substernal pain and a mass in the abdomen, all of 3 months' duration. There was slight anemia and a differential count showed a few myelocytes and normoblasts. Pleural fluid contained "lymphosarcoma" cells. He died on October 23, 1943.

A group of lymph nodes removed from the groin on September 14, 1943, measured 3 cm. in diameter. They were well encapsulated, moderately firm and pinkish gray. Microscopically, the normal architecture was completely replaced by a diffuse infiltration of large, and in fewer numbers, small, lymphocytes (Fig. 11). The former showed mitosis and occasionally some fragmentation. Thick-walled capillaries with an increase of surrounding collagen were inconspicuous. Scattered sparsely throughout the nodes were a few faintly stained, ill defined reticulum cells. There was no other pleomorphism.

Autopsy performed on October 23, 1943, showed considerable emaciation. The axillary, clavicular and inguinal lymph nodes measured as much as 5 cm. in diameter, while those of the mediastinum and abdomen were about half that size. They were all moderately firm, pinkish gray and showed no necrosis or hemorrhage. The spleen weighed 290 gm. and the liver, 1390 gm. The latter contained several nodules of light pinkish gray tissue which measured as much as 5 cm. across. There were similar but smaller nodules in each lower lobe of the lungs. In addition, the left pleura was studded with many small, flat tumor masses and the corresponding pleural cavity contained 1000 cc. of blood-tinged fluid. The kidneys contained ill defined, light gray foci measuring less than 5 mm. across. The remaining organs were essentially normal.

Microscopical sections of most of the lymph nodes showed a complete replacement of the normal architecture with a mixture of large and small lymphocytes, plasma cells, polymorphonuclear leukocytes, a few eosinophils and many Sternberg-Reed cells (Fig. 12). There were, in addition, large phagocytes containing nuclear fragments. In some nodes nearly all of the cells were large lymphocytes, scattered amongst

which were very occasional phagocytes and Sternberg-Reed cells. The reticulum in these nodes was increased. Sections from the liver, kidneys, lungs and pleura showed lesions typical of Hodgkin's disease.

Summary. Sections of the peripheral nodes obtained for biopsy showed lymphosarcoma, whereas sections of most of the internal nodes examined at autopsy 1½ months later disclosed Hodgkin's disease. A few, however, still possessed some features of a lymphosarcoma.

Case 5

A white woman, 65 years of age, was admitted to the hospital in August, 1943, complaining of weakness, generalized lymphadenopathy and loss of weight for 2 years, and of an enlarged, tender spleen for 2 months. A severe progressive anemia preceded death in December, 1943.

Several matted axillary lymph nodes, measuring 3 cm. in diameter, were removed on September 9, 1943. They were well encapsulated and uniformly pinkish brown. Microscopical sections disclosed a complete replacement of the normal architecture with mostly large, but also a few small, lymphocytes (Fig. 13). Scattered throughout the parenchyma were varying numbers of reticulum cells which were either indistinct and united with the fibrils, or were separated off and either round or polygonal. Their cytoplasm was pink and abundant and their nuclei were evenly stained. They did not resemble Sternberg-Reed cells. There was no other pleomorphism.

Autopsy performed on December 24, 1943, disclosed considerable emaciation and jaundice. The lymph nodes in the mediastinum and throughout the abdomen were enlarged to 3 cm. in diameter, but showed little matting. They were firm and, except for central areas of necrosis in the thoracic nodes, uniformly pinkish gray. The spleen weighed 1490 gm. It was firm, dull reddish gray and contained numerous grayish nodules measuring as much as 3 mm. in diameter. The marrow of the lower thoracic and lumbar vertebrae was dull red and disclosed many irregular gray foci measuring up to 0.5 cm. in diameter. The left pleural cavity contained 1500 cc. of clear straw-colored fluid. The rest of the organs, including the liver and kidneys, were grossly normal.

Microscopically, the lymph nodes showed typical lesions of Hodgkin's disease (Fig. 14). Their normal architecture was completely replaced with varying numbers of large lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and many Sternberg-Reed giant cells. Phagocytes containing nuclear fragments and hemosiderin were quite prominent. There were areas of necrosis and slight to moderate fibrosis. The spleen and bone marrow were diffusely involved with a similar process. There was an infiltration of lymphocytes,

plasma cells, polymorphonuclear leukocytes and occasional eosinophils between the liver cords. The latter were either partially or completely destroyed. The portal areas contained an increase of fibrous tissue and lymphocytes.

Summary. Biopsy of an axillary lymph node disclosed a lymphosarcoma with some added features of a reticulum cell sarcoma. Examination of the internal lymph nodes at autopsy 3½ months later showed Hodgkin's disease.

Case 6

A white man, 53 years old, was admitted to the hospital in December, 1942, complaining of weakness and lymphadenopathy of recent origin. He had enlargement of the spleen and liver. X-ray therapy was followed by some improvement until March, 1943, when weakness, anorexia, loss of weight and a diffuse inflammation of the skin of the lower abdomen and upper thighs became incapacitating. He died on May 5, 1943.

A group of nodes from the left inguinal region removed on December 12, 1942, measured 4 by 2 cm. The nodes were matted together and moderately firm. Cut surfaces showed pinkish gray tissue streaked with very recent hemorrhage. Microscopically, there was a complete, thin, peripheral rim of densely packed, large and small lymphocytes. The former showed many mitoses. Occasionally a faintly stained reticulum cell was present in the background. In the central portions of the nodes the cells were less numerous (Fig. 15). They were larger than large lymphocytes but smaller than the typical reticulum cells seen in case 1. Their nuclei were usually evenly stained although some were in a state of mitosis. Their cytoplasm was pink and prominent. No other cell types were present.

Autopsy on May 5, 1943, showed enlargement of the cervical, axillary, inguinal, mediastinal and abdominal lymph nodes to as much as 3 cm. in diameter. They were matted together and firm. Their cut surfaces were uniformly pinkish gray and showed no areas of hemorrhage or necrosis. The liver weighed 2740 gm. The portal areas were prominent and contained bands of gray tissue measuring 1 to 2 mm. across. The left pleural cavity contained 1700 cc. of straw-colored fluid. The rest of the organs, including the spleen and bone marrow, were grossly normal.

Microscopically, some of the nodes were identical with those removed on December 12, 1942, and described above, with the exception that the peripheral rim of cells was absent and all of the cells were similar to those seen in the central portions of those nodes. There were other lymph nodes, however, which showed considerable pleomorphism (Fig. 16). In addition to the large cells described above there were polymorphonuclear leukocytes, plasma cells, occasional eosinophils

and many Sternberg-Reed giant cells. Infiltrations of similar cells were found in the spleen, the portal canals of the liver, the cortices of the kidneys, the skin, and in and about the bronchi.

Summary. Biopsy in December, 1942, showed a transition between a large cell lymphosarcoma and reticulum cell sarcoma. Autopsy 6 months later disclosed, in addition, typical Hodgkin's disease.

Discussion

When a primary tumor in a lymph node is a typical lymphosarcoma, Hodgkin's disease, or reticulum cell sarcoma, there is no difficulty in making a definite diagnosis. This is not so, however, when the cellular composition is not of the type ordinarily designated as characteristic of one of the three diseases and often one encounters such combinations of cells that it is virtually impossible to label the tumor definitely. Furthermore, these combinations can be explained only by considering the three diseases as not only closely related but as having a common neoplastic origin, differentiating along one line in one lymph node or a portion of that node and along another line in another node or a portion of the same node. Or, it may be that for a time differentiation proceeds in one direction in all of the nodes and then as a result of treatment or spontaneously it proceeds in an entirely different direction.

The above considerations are well supported by the cases presented. Thus in case 1 a diagnosis of Hodgkin's disease was first made and 1 year later a diagnosis of lymphosarcoma was made on another lymph node. Two and one-half years later a third node again showed typical Hodgkin's disease while autopsy 1⅓ years later showed the changes of lymphosarcoma in some nodes, of Hodgkin's disease in others and of reticulum cell sarcoma in still others. It might well be argued that, because of the presence in the latter nodes of a few lymphocytes and occasional polymorphonuclear leukocytes, the process is a sarcomatous type of Hodgkin's disease. Yet if such a node were seen as a biopsy specimen one would not be justified in making any other diagnosis than that of reticulum cell sarcoma.

In case 2 the lesion seen on May 9, 1940, was in all respects the typical granulomatous form of Hodgkin's disease, abounding not only in eosinophils but also in Sternberg-Reed cells. A year and one-half later another biopsy showed a diffuse infiltration with regular large lymphocytes exhibiting much mitotic activity and only occasional polymorphonuclear leukocytes and a few underlying reticulum cells. Without the presence of Sternberg-Reed cells, which, according to Potter,[16] is the one specific feature of Hodgkin's disease, a diagnosis other than lymphosarcoma could not have been made. Certainly such a diagnosis

cannot be excluded because of the presence of regular reticulum cells in the background for they are present in normal lymph nodes. The same combination of large and small lymphocytes with a few reticulum cells scattered throughout the sections and the absence of eosinophils and Sternberg-Reed cells in cases 3, 4 and 5 accounted for the diagnosis of lymphosarcoma in the original specimens taken for biopsy. These cases might just as easily have continued to show the same picture or have been transformed into an outright reticulum cell sarcoma instead of developing into Hodgkin's disease. The alternative would be to call them atypical Hodgkin's disease which to us seems to be merely playing with words and evading the fundamental issue.

Finally, case 6 illustrates a neoplasm with cells larger than a large lymphocyte and smaller than an ordinary reticulum cell. The first biopsy showed, in addition, a peripheral rim of neoplastic tissue composed of typical large and small lymphocytes. Into what category can such a combination be placed? The lesion is neither an outright lymphosarcoma nor is it an outright reticulum cell sarcoma. It is half way between. Yet at autopsy 6 months later, while most of the nodes were composed of the large cells previously referred to, there were some which showed considerable cellular pleomorphism together with typical Sternberg-Reed cells. In these the changes were surely those of Hodgkin's disease. Thus, as already stated, it appears that the only logical explanation is to regard lymphosarcoma, Hodgkin's disease and reticulum cell sarcoma as ultimately arising from a common stem cell—the reticulum cell—and then according to further differentiation or lack of differentiation forming either combinations of these diseases or distinctly outright pictures of one type or the other.

If we accept the fact that these variations in cytological morphology are traceable to changes in differentiation of one cell type—the reticulum cell—then we must consider the presence of at least two stimulators that can bring about such changes in differentiation. These tumors are highly complex in cellular composition; therefore, if they are related to lymphosarcoma, part of the cellular proliferation that occurs in them must be brought about by the same factor that accounts for proliferation in the lymphosarcomas. Proliferation arising because of this stimulator would be lymphoid. The presence of myeloid cells such as neutrophils, eosinophils and possibly Sternberg-Reed cells is an indication of a second stimulator, one that would bring about myelopoiesis.

Thus, the variability in cellular components in these tumors may be accounted for by the local and/or general variations in activity of the myeloid and lymphoid stimulator substances. It has been shown that there are two such substances, one of which is specific for stimulating

the proliferation of myeloid cells, the other is specific for stimulating the proliferation of lymphoid cells.[15] These substances appear to be mutually reciprocal in action, *i.e.*, the myeloid stimulator causes the maturation of lymphoid cells and the lymphoid stimulator causes the maturation of myeloid cells. These substances have been found to occur in increased amounts in nearly equal proportions in the urine of patients with Hodgkin's disease and monocytic leukemia. Both are present, also, in the lipids of normal beef liver.[17] Crude extracts of the urine of patients with Hodgkin's disease and crude extracts of the lipids of normal beef liver produced pleomorphic lesions in the organs of guinea-pigs when such material was injected into these animals. Chemical separation of these extracts into carbinols and noncarbinols resulted in two equally potent fractions. The carbinols in each instance elicited a response of increased lymphopoiesis and the noncarbinols that of increased myelopoiesis. Noncarbinols and carbinols obtained from the urine of patients with chronic leukemia were given simultaneously to guinea-pigs and the response was similar to that obtained when crude unseparated extracts were given. The lesions elicited were not those typical of Hodgkin's disease, but in the livers, lungs, adrenals, kidneys and spleens of these animals there was hyperplasia of the lymphoid elements and a stimulation of the reticulum cells. The reticulum cells occasionally had more than one nucleus. Frequently there were a large number of eosinophils among the lymphoid cells and a few neutrophils. The lesions in the liver frequently were around blood vessels. An increased number of fibroblasts was seen in many of the lesions.

Extracts of the urine of seven patients with Hodgkin's disease were used in this work. Each gave about the same biological response with one exception, namely, case 1 of this report. Urine from this patient, obtained and extracted at the time that the diagnosis of lymphosarcoma was made on biopsy material, gave a response in the guinea-pig of increased lymphopoiesis only.

This work and the changing picture presented by these cases lead us to question how we can best describe Hodgkin's disease. Healing and regression that involve the proliferation, maturation and destruction of many of the cellular elements of the blood occur in Hodgkin's disease, and this process continually repeats itself as the neoplasm grows. A system of three parts is necessary for the activation of this process: (1) the presence of mesenchymal stem cells; (2) an excess of the two substances capable of stimulating proliferation of the stem cells so that myeloid and lymphoid proliferation occur simultaneously; and, (3) such substances should not be in such excess as to preclude

their reciprocal action, but would allow for a certain amount of maturation to take place. This system allows typical Hodgkin's disease to develop. It is possible that locally there might be an excess of one substance and therefore a few nodes would be typical of lymphosarcoma or the bone marrow would be hyperactive in myelopoiesis. A general excess of one substance might occur for a short time and later be followed by an excess of both. Either of these explanations might account for the variations encountered in these cases. If the excess of both substances were so great locally that stimulation of cellular proliferation without maturation occurred, then the result would be reticulum cell sarcoma. If equally great excesses were evidenced generally, then monocytic leukemia would be the result.

Other factors are probably involved, but for the most part this system seems to explain not only typical Hodgkin's disease, but also the variations of the disease that are reported here.

Summary

Six cases are presented that at one time during the lives of the patients were diagnosed as Hodgkin's disease and at another time as lymphosarcoma and that at autopsy showed various combinations of Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma. These various combinations can be explained only by considering the three diseases as arising from a common stem cell—the reticulum cell—and then differentiating in one direction or another according to the amount and type of stimulation. Proliferation arising because of an excess of the lymphoid stimulator gives rise to a lymphosarcoma while that due to an excess of both lymphoid and myeloid stimulators gives rise to Hodgkin's disease, providing the stimulation is accompanied by maturation, or to reticulum cell sarcoma when unaccompanied by maturation. Combinations of these diseases result when the specific stimulators are not uniformly distributed throughout the organs or when a temporary excess of one is followed by a temporary excess of the other.

We are indebted to Dr. C. J. Bucher for allowing us to use the surgical material and to Dr. Joseph Stasney who performed the autopsy in case 3.

REFERENCES

1. Gibbons, H. W. The relation of Hodgkin's disease to lymphosarcoma. *Am. J. M. Sc.*, 1906, **132**, 692–704.
2. Coley, W. B. Hodgkin's disease a type of sarcoma. *N. Y. M. J.*, 1907, **85**, 577–583.
3. Oliver, J. The relation of Hodgkin's disease to lymphosarcoma and endothelioma. *J. M. Research*, 1913–14, **29**, 191–207.
4. Mallory, F. B. The Principles of Pathologic Histology. W. B. Saunders Co., Philadelphia, 1914, p. 326.

5. Richter, M. N. Generalized reticular cell sarcoma of lymph nodes associated with lymphatic leukemia. *Am. J. Path.*, 1928, **4**, 285–292.
6. MacMahon, H. E., and Parker, F., Jr. A case of lymphoblastoma, Hodgkin's disease and tuberculosis. *Am. J. Path.*, 1930, **6**, 367–380.
7. Craver, L. F. Clinical manifestations and treatment of leukemia. *Am. J. Cancer*, 1936, **26**, 124–136.
8. MacCarty, W. C. A cytologic study of Hodgkin's disease, lymphosarcoma and lymphatic leukemia. *J. Cancer Research*, 1930, **14**, 394–399.
9. Levin, I. Lymphoma malignum (Hodgkin's disease) and lymphosarcoma. Pathogenesis, radiotherapy and prognosis. *J. A. M. A.*, 1931, **96**, 421–426.
10. Ginsburg, S. Lymphosarcoma and Hodgkin's disease: biologic characteristics. *Ann. Int. Med.*, 1934–35, **8**, 14–36.
11. Cohn, S., and Richter, M. Modern views on Hodgkin's disease. *M. Rec.*, 1938, **148**, 243–246.
12. Warthin, A. S. The genetic neoplastic relationships of Hodgkin's disease, aleukemic and leukemic lymphoblastoma, and mycosis fungoides. *Ann. Surg.*, 1931, **93**, 153–161.
13. Sugarbaker, E. D., and Craver, L. F. Lymphosarcoma. A study of 196 cases with biopsy. *J. A. M. A.*, 1940, **115**, 17–23; 112–117.
14. Callender, G. R. Tumors and tumor-like conditions of the lymphocyte, the myelocyte, the erythrocyte and the reticulum cell. *Am. J. Path.*, 1934, **10**, 443–465.
15. Miller, F. R., and Turner, D. L. The action of specific stimulators on the hematopoietic system. *Am. J. M. Sc.*, 1943, **206**, 146–158.
16. Potter, E. L. Hodgkin's disease, with special reference to its differentiation from other diseases of lymph nodes. *Arch. Path.*, 1935, **19**, 139–158.
17. Turner, D. L., and Miller, F. R. Specific stimulators of hematopoiesis from beef liver. *Proc. Soc. Exper. Biol. & Med.*, 1943, **54**, 177–179.

[*Illustrations follow*]

DESCRIPTION OF PLATES

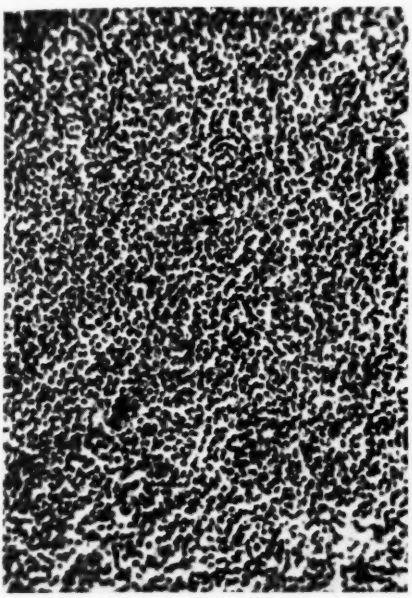
PLATE 37

FIG. 1. Case 1. Section of a lymph node removed in May, 1941, showing a diffuse infiltration with lymphocytes. Hematoxylin and eosin stain. × 200.

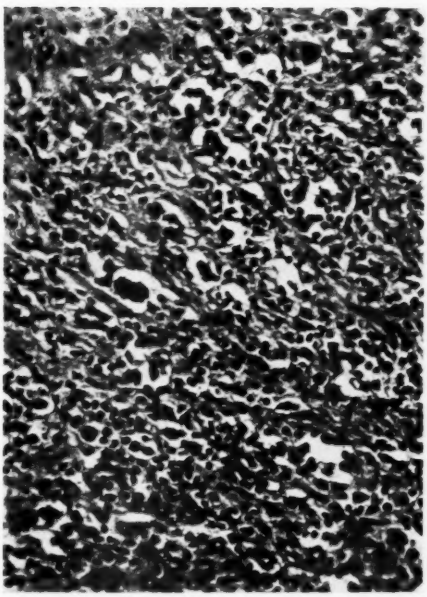
FIG. 2. Case 1. Section of a lymph node removed in September, 1943, showing moderate fibrosis, and a diffuse infiltration with lymphocytes, plasma cells, a few polymorphonuclear leukocytes, scattered eosinophils and typical Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.

FIG. 3. Case 1. Section of a lymph node obtained at autopsy in December, 1943, showing a slight increase in fibrous tissue and a diffuse infiltration with mature lymphocytes. Hematoxylin and eosin stain. × 200.

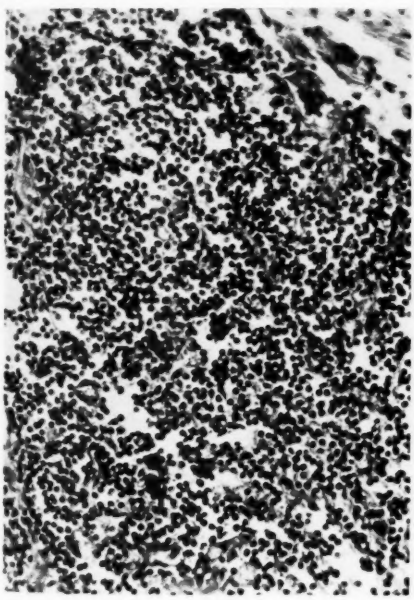
FIG. 4. Case 1. Section of a lymph node obtained at autopsy in December, 1943, showing considerable fibrosis and an infiltration with lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.



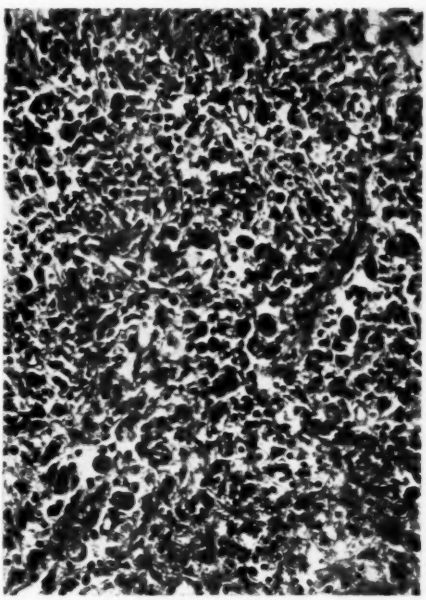
1



2



3



4

Herbut, Miller and Erf Relation of Hodgkin's Disease

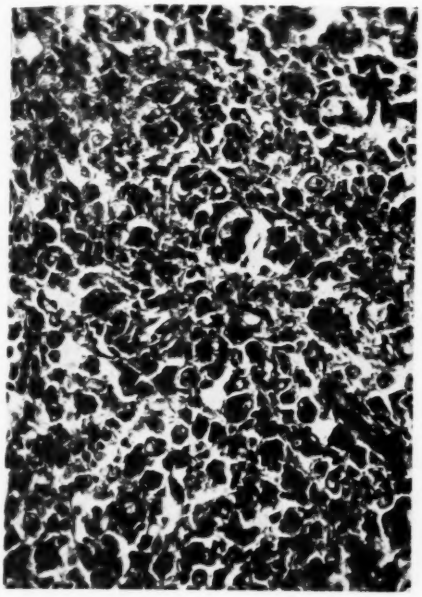
Plate 38

Fig. 5. Case 1. Section of a lymph node obtained at autopsy in December, 1943, showing an almost pure proliferation of reticulum cells. There are also present a few scattered lymphocytes. Hematoxylin and eosin stain. × 200.

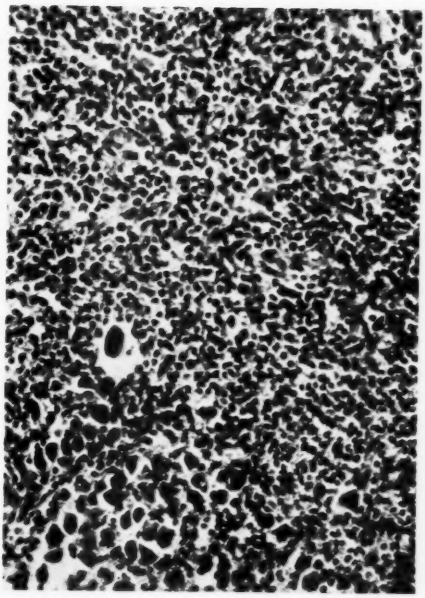
Fig. 6. Case 2. Section of a lymph node removed in May, 1940, showing a diffuse replacement of the normal architecture with eosinophils; fewer lymphocytes, plasma cells and polymorphonuclear leukocytes, and clusters of typical Sternberg-Reed cells. Hematoxylin and eosin stain. × 200.

Fig. 7. Case 2. Section of a lymph node removed in December, 1941, showing mostly large lymphocytes; scattered small lymphocytes and a very occasional, poorly stained reticulum cell in the background. Hematoxylin and eosin stain. × 200.

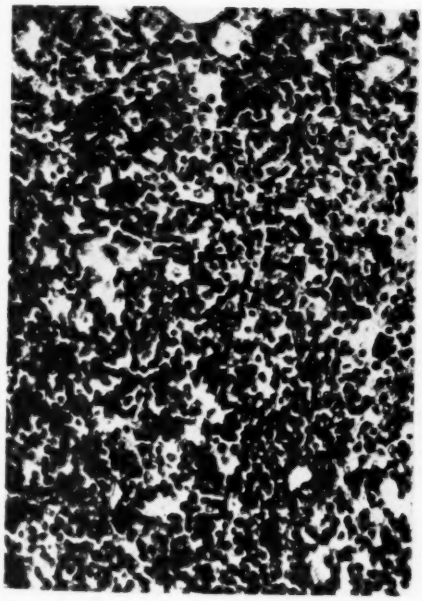
Fig. 8. Case 2. Section of a lymph node obtained at autopsy in December, 1942, showing a diffuse infiltration with large lymphocytes, lesser numbers of small lymphocytes and only an occasional polymorphonuclear leukocyte. Hematoxylin and eosin stain. × 200.



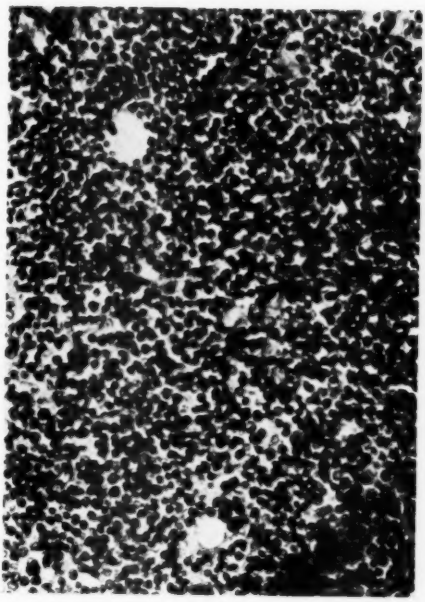
5



6



7



8

Herbut, Miller and Erf

Relation of Hodgkin's Disease

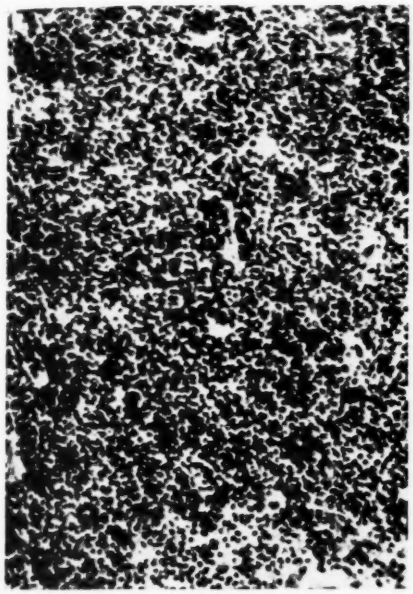
PLATE 39

FIG. 9. Case 3. Section of a lymph node removed in April, 1942, showing a dense infiltration with mature lymphocytes. In the background there are a few ill defined, faintly stained reticulum cells. Hematoxylin and eosin stain. × 200.

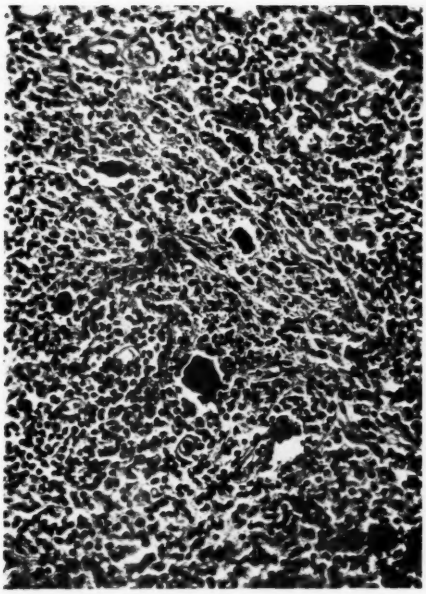
FIG. 10. Case 3. Section of a lymph node obtained at autopsy in September, 1943, showing a slight increase in reticulum and a diffuse infiltration with large and small lymphocytes, polymorphonuclear leukocytes, plasma cells and typical Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.

FIG. 11. Case 4. Section of a lymph node removed in September, 1943, showing a diffuse infiltration with lightly and darkly stained large and small lymphocytes. Occasionally the nuclei of the former are fragmented. Hematoxylin and eosin stain. × 200.

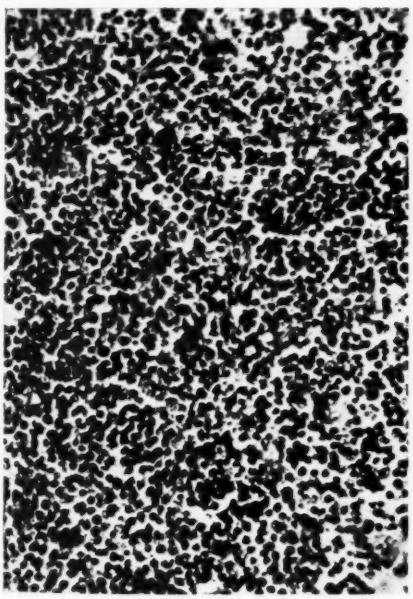
FIG. 12. Case 4. Section of a lymph node obtained at autopsy in October, 1943, showing large and small lymphocytes, plasma cells, polymorphonuclear leukocytes, a few eosinophils and many Sternberg-Reed giant cells. There are also several large phagocytes laden with nuclear fragments. Hematoxylin and eosin stain. × 200.



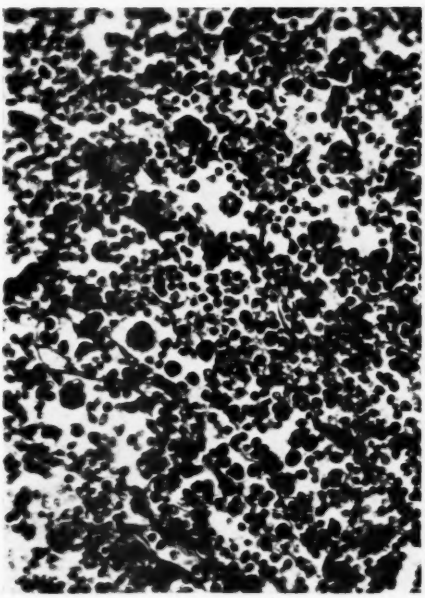
9



10



11



12

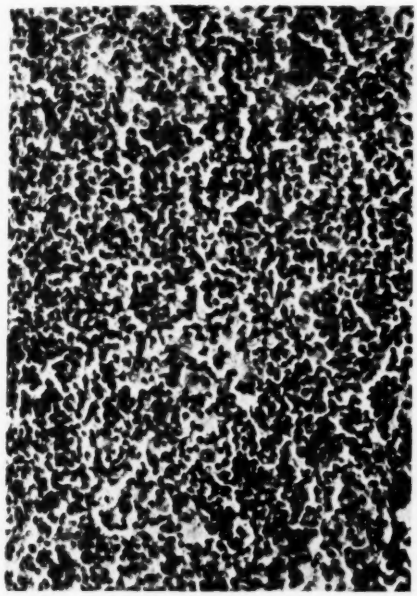
Plate 40

Fig. 13. Case 5. Section of a lymph node removed in September, 1943, showing a diffuse infiltration with large and small lymphocytes. In the background there are scattered, faintly stained and ill defined reticulum cells. Hematoxylin and eosin stain. × 200.

Fig. 14. Case 5. Section of a lymph node obtained at autopsy in December, 1943, showing a diffuse infiltration with large lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and many Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.

Fig. 15. Case 6. Section of a lymph node removed in December, 1942, showing a diffuse infiltration with large cells which are larger than large lymphocytes but smaller than typical reticulum cells. Hematoxylin and eosin stain. × 200.

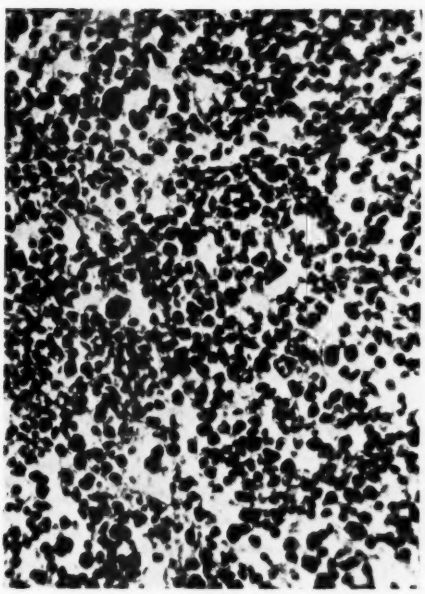
Fig. 16. Case 6. Section of a lymph node obtained at autopsy in May, 1943, showing large cells in Figure 15 and also polymorphonuclear leukocytes, plasma cells, occasional eosinophils and several Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.



13

14

15



16

Herbut, Miller and Erf

Relation of Hodgkin's Disease

ARREST AND REPAIR IN EXPERIMENTAL ENDOCARDITIS LENTA *

WARD J. MAC NEAL, M.D., ANNE BLEVINS, R.N., MARCELLO R. PACIS, M.S., and ALICE E. SLAVKIN, B.S.

(From the Department of Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University, New York, N. Y.)

In previous papers we have shown some of the changes observed very early [1] after rabbits had been inoculated intravenously with streptococci isolated from the blood of human patients with endocarditis and also some of the more advanced lesions [2] when the vegetations of the valve leaflets had attained large size. From these studies it has been possible to gain an idea of the successive stages in this infectious disease as it attacks the cardiac valves in the experimental animal and progresses to a fatal termination. It should be acknowledged, however, that only fixed pictures or stages of the process have been observed and that the relation of one stage to another has been surmised rather than actually seen, a defect which is inherent in purely morphological studies and not fully escaped even when such studies have been made upon experimental material, in which the individual experiment has been terminated at will.

When we undertook to examine the instances of apparent arrest and repair of the lesions in these experimental animals, even greater difficulties were encountered. That fibrous thickening and deformity, observed in an animal sacrificed several weeks or months after initiation of experimental inoculations, might be properly regarded as evidence that these alterations had resulted from the repair of a previously active lesion of the experimental disease is an assumption rather than an observed fact and should be accepted with reserve and even with suspicion until repeated observations of similar changes have supplied adequate confirmation. The probable nature of such an assumption is afforded some support by the recorded behavior of the animal and the laboratory tests made during the course of the disease, such as changes in temperature, in body weight, in the heart sounds and especially the results of cultures of the blood. The problem is much the same as that presented by the necropsy when a good clinical record of the case is available.

In general, experimental endocarditis, once established in the rabbit, tends to progress to a fatal termination. Nevertheless, one sometimes observes evidence of local arrest and local healing. Spontaneous re-

* Aided in part by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry, American Medical Association, and by a grant from the United Hospital Fund of New York City. Reproduction of the colored illustrations has been made possible by the William Cotton Damon Research Fund.

Received for publication, May 6, 1944.

covery in the rabbit appears to take place rarely if at all, perhaps about as often as it may occur in the human disease. By the application of therapeutic measures we have been able to alter the natural course of the infection. In many instances administration of large doses of serum, vaccines, sulfonamides and other chemicals was followed by the production of large valvular vegetations, extensive myocardial damage and early death. In recent years, however, greater therapeutic success has been attained in a few animals.

In the arrest of the disease and repair of the injured structures, various biological mechanisms are concerned. At the very onset, when the initial shower of streptococci has entered the circulating blood, there is evidence of a rapid phagocytosis of these bacteria by wandering cells and by the endothelial cells lining the blood vessels in general. The vast majority of the bacteria are killed and digested by the phagocytic cells without leaving any persistent anatomical alteration at the site of their destruction. Even after prolonged streptococcemia the liver ordinarily shows no recognizable structural alteration, although there can be no doubt that myriads of streptococci have been digested there. In the spleen, there is marked enlargement due to increase of all the elements, but especially to congestion, and after prolonged infection there is evidence of progressive fibrosis about the follicles. In the lungs there is early thickening of all the alveolar septa with formation of septic capillary thrombi and also a conspicuous endarteritis with edematous thickening, perivascular edema and hemorrhages along the branches of the pulmonary arteries. The lungs receive and destroy large numbers of the injected streptococci, which are abundantly held in the pulmonary capillaries. In the heart the bacteria are ordinarily fixed by the endocardium and in the myocardial capillaries to a lesser extent, but while the spleen, liver and lungs destroy the bacteria, usually without trace, the lining endothelium of the cardiac cavities and of the myocardial vessels often fails to exterminate completely the streptococci deposited here. It seems that the pressure of contact of the valve surfaces during the movement of the heart favors the persistence of the bacteria at the contact sites. In the myocardium, endothelial phagocytosis of the streptococci is associated with swelling of the endothelial cells concerned and this in turn diminishes the vitally essential supply of blood through the myocardial capillaries. Apparently on this account the bacteria tend to survive more readily in these situations.

Once there has been produced a mass of infected fibrin on the valve leaflet, the bacteria tend to grow in the fibrin as compact colonies and in these colonial masses they are evidently able to produce enough toxic substances to keep at a distance any phagocytic cells. As long as the

bacteria here are able to multiply rapidly in masses they defy all efforts of the host to destroy them.

It appears, however, that the proliferation of these streptococci may be influenced particularly by antagonistic substances present in the blood. Some of these antibacterial substances are produced by the tissues of the host. In untreated patients the blood culture may at one time develop a visible growth only after 3 or more days and in a later blood culture the growth may be distinct in less than 24 hours. The indicated bacteriostatic effect is even more evident in patients subjected to treatment with anti-infectious therapeutic agents. Evidently an antibacterial effect may be exerted upon colonies in the endocardial vegetations as well as upon cocci free in the circulating blood and their progeny in the culture flask. Is it possible that such agents in the circulating blood may influence the infected vegetations to such a degree that the bacteria no longer produce poisons in sufficient amounts to keep the healing elements at bay? We believe that this question may be answered in the affirmative. In some of the experimental animals, various stages of arrest and repair have been observed, sometimes in control animals but more often in treated animals.

The injury inflicted upon the streptococci is manifested by various degrees of bacteriolysis revealed by alterations in size, form and arrangement, by abnormal staining of the bacteria, and by their fragmentation and dissolution; also by the more intimate approach of phagocytic cells and the actual ingestion by them of the streptococci in the vegetation. Furthermore, the mass of infected fibrin no longer repels the advance of endothelial and fibroblastic cells, but now permits these healing elements to penetrate and surround it so as to wall in the crippled bacterial colonies and eventually produce a scar at the site of their extermination. Individual examples of arrest and repair may serve to illustrate the varied processes observed in the endocardium.

Experimental Observations

Rabbit 166, series 4B. Daily intravenous doses of living culture of Streptococcus faecalis (enterococcus), for 13 days; repeated positive blood cultures; abnormal heart sounds; treated with specific bacteriophage and thiobismol; death on the 30th day; large active mitral vegetation with local healing; healed aortic valvular lesion.

Rabbit 166, weighing 1665 gm., was inoculated with *Streptococcus viridans,* strain L 114-1, originally isolated from the blood of a patient who died of typical endocarditis lenta. The culture had been subsequently passaged in rabbits. The animal received intravenous injections of a heavy suspension of the living bacteria: 0.25 cc. on February

16, 1943, and 0.50 cc. daily from February 17 to March 1, inclusive, when inoculations were discontinued. The weight had fallen to 1490 gm. on February 28 and the blood culture taken on February 24, about 24 hours after the preceding bacterial inoculation, had given positive growth. Auscultation of the heart had been negative. Blood cultures taken on March 4, 11 and 17 were all positive. Auscultation was negative until March 3, at which time a faint mitral murmur was recorded. Subsequently, abnormal sounds were recorded on March 4, 5, 8, 9, 10, 12, 15, 17 and 18. On March 15, 16 and 17 an aortic murmur was diagnosed and on March 3, 4, 17 and 18 there was recorded a mitral impurity.

Intravenous therapy was instituted on February 17, the day after initial inoculation. At about 10:30 A.M. the animal was given 2 mg. of thiobismol and 10 cc. of specific asparagine bacteriophage intravenously, and in the afternoon a second intravenous dose of 10 cc. of bacteriophage. This daily program was continued through February 23. Then it was supplemented by added intramuscular injections of 3 mg. of thiobismol, given in the afternoon on February 24, 25, 26 and 27; March 3, 4 and 5; March 9, 10 and 11; and March 15, 16 and 17. The rabbit weighed 1545 gm. on March 7 and 1535 gm. on March 15. On March 18 all normal heart sounds were replaced by a blowing mitral systolic murmur. The animal died at 3:15 P.M. and the body was fixed by arterial perfusion with Helly's formalized Zenker's solution at 3:30 P.M. This fixative obscured to some extent the gross pathological changes but produced most excellent fixation for the study of minute histological detail, including the bacteria. In the heart there was a ragged vegetation about 6 mm. in diameter completely occluding the mitral orifice. The tricuspid valve appeared thickened, the aortic valve negative. There were many petechial hemorrhages in the lungs, marked passive congestion of the liver, and the spleen was much enlarged. A gross photograph of the heart is shown in Figure 1. Part of a section through the mitral vegetation is represented in Figures 2, 3 and 4. The vegetation is large and in part resembles those seen in the unrestricted progressive lesions. However, its structure is not uniform and in places one can recognize that the defensive forces of the rabbit have to some extent overcome the bacterial invaders.

Two phenomena are of special interest. In the cell-free fibrin of the vegetation there are rounded bacterial colonies in which only part of the microbes take the bacterial stain in a normal manner and a considerable part of the colony consists of débris in which faintly tinted bacterial cells may still be recognized. These colonies have evidently been caught by the fixing agent in a stage of progressive lysis and they

illustrate a process of bacterial destruction in which phagocytosis by wandering cells or fixed endothelium is not directly concerned. A group of three such colonies is represented in Figure 5. One may, perhaps, speculate in regard to the possible participation of various antibacterial factors, such as the thiobismol, the injected bacteriophage, lytic agents produced by the rabbit tissues or autolytic ferments of the streptococci themselves. Further experimental exploration may aid in elucidation of the question. At present we wish merely to present the phenomenon as one feature in the arrest of the disease.

A second kind of defense activity is represented in Figure 6. At this site the body cells have been able to approach the bacterial groups and have given rise to purulent exudate in which many of the bacteria have been ingested and some of them partly digested within phagocytic cells. This phagocytosis is of a different order from that seen in the initial localization of the streptococci in the endothelial cells of the endocardium, where the bacteria in the circulating blood, washed relatively free of soluble materials, may be taken up by the cells much as would be inert particles of carbon. Here the bacteria have already established themselves and begun to grow and cause local necrosis. The formation of cellular exudate and actual phagocytosis of the bacteria would seem now to require some special influence unfavorable to the bacteria, probably some agent in solution, or else some kind of acquired ability of the body cells to resist the bacterial poisons and to approach near to the growing streptococci.

Associated with this phenomenon of purulent exudation and phagocytosis there is a third manifestation, namely, the proliferation of fibroblasts and endothelial cells in the immediate vicinity and the extension of these tissue elements around and into the necrotic fibrin and purulent exudate to encapsulate and to organize the lesion as the bacteria are exterminated. An early stage of this defensive mechanism is illustrated in Figure 7, showing a bacterial colony on the mitral valve. In this one experimental animal there are represented these three main types of alteration. They tend to overcome the streptococci and to bring about their isolation and destruction and also to remove the necrotic material and repair the regional defect by construction of fibrous stroma and a covering endothelial coat. The arresting processes did not succeed and the animal did not survive.

Rabbit 145, series 2B, was inoculated intravenously with *Streptococcus viridans* (enterococcus), strain L 114-1, daily, on December 25 to 29, inclusive, and died on December 29. Perfusion fixation was carried out immediately. The general infection was in an active stage and had caused destructive lesions in the myocardium as well as early pro-

gressive vegetation on the mitral valve. A section through a swollen mitral leaflet is shown in Figure 8. At this site the bacteria have penetrated into the substance and have established a colony beneath the endothelium and this colony is surrounded by a loosely assembled wall of wandering cells. Figure 9 shows this colony and the adjacent structures in color. There are scattered streptococci in the substance of the valve about the bacterial colony and even at a considerable distance from it. Some cocci can be recognized within a polynuclear leukocyte about 50 μ from the endothelial surface at the right of the figure. Many of the cocci near the colony have been taken up by phagocytic cells and it would seem that the bacterial penetration may have come about through transport by wandering cells or possibly by movement of plasma in the intercellular spaces of the stroma after injury to the endothelial covering of the valve. A part of the bacterial colony at higher magnification is represented in Figure 10. Many of the streptococci at the periphery of the colony have failed to retain the Gram stain and some of the cocci within the phagocytic cells appear to be partly digested. In this untreated animal there is evidence of unsuccessful defense by the mechanisms of bacteriolysis, phagocytosis and partial encapsulation of the invading bacteria.

Rabbit 593, series 14A, was inoculated by intravenous injection of bacterial suspension, *Streptococcus viridans* (salivarius), strain CC, daily for 8 days, and was then allowed to remain without further inoculation and without treatment until death on the 18th day, when the body was immediately fixed by arterial perfusion. Various stages in healing of local lesions have been observed in untreated animals from time to time and these have been found most frequently in the right heart, on the mural endocardium, the tendinous cords, or the tricuspid leaflets. In rabbit 593, however, there was a well encapsulated, small, round vegetation situated near the free margin of the mitral leaflet (Fig. 11). This evidently developed in the usual way to produce a mass of infected fibrin attached to the auricular face of the leaflet and subsequently became encapsulated by growth and extension of endothelial and fibroblastic cells around and into the fibrin near the surface. These cells have also extended inward to approach the "unhappy" bacterial colonies and to ingest some of the bacteria. The periphery of the encapsulated vegetation is shown in Figure 12. Evidently the bacterial proliferation has been restrained by something in the animal's blood so that the living cells have been able to take possession of the superficial layers of the fibrin and to grow inward to come into intimate contact with the bacterial colonies. Again, however, the defense mechanisms have not been sufficiently powerful to exterminate the bacterial

invaders and, in fact, at other parts of the mitral valve of this animal more progressive lesions were still evident. In spite of a partial defense, the disease went on to a fatal termination.*

These few examples of the many animals in which we have observed partial arrest and partial healing of endocardial lesions may serve to introduce a consideration of some of the rabbits in which complete arrest and healing have occurred and may help in the undertaking and interpretation of the scars and deformities observed in these recovered animals.

Rabbit 392, series 5B, inoculated with an enterococcus strain of *Streptococcus viridans* and treated with specific bacteriophage and thiobismol, is an example of apparently complete arrest of the disease. A photograph of a gross section through the mitral orifice is shown in Figure 13. We regard the deformity of the mitral leaflets and the smooth, irregularly rounded nodules as sequelae of healed vegetations. Microscopical examination has failed to disclose any bacteria and the healing is evidently complete. Similar observations have been made in several rabbits, of which no. 133, series 2B, will be considered in more detail.

Rabbit 133, series 2B. Daily intravenous inoculations of living culture of Streptococcus faecalis for 6 days; repeated positive blood cultures for subsequent 12 days; later blood cultures, negative; treatment with bacteriophage and neoarsphenamine; in apparent health when sacrificed on 47th day; mitral valve healed with deformity.

Rabbit 133, series 2B, weighed 1500 gm. on December 23, 1942. It was inoculated with suspensions of bacterial culture, strain L 114-1, an enterococcus isolated from the blood of fatal human endocarditis and subsequently passaged through a rabbit. The inoculations were given intravenously, 2 cc. daily, December 25 to 30, inclusive, to a total amount of 12 cc., and then discontinued. The weight fell to 1355 gm. on December 30. Blood culture taken on December 31 was positive, as were subsequent blood cultures taken on January 6 and 11. Later blood cultures taken on January 19 and 25, and on February 3 and 9 remained sterile. The lowest weight observed was 1330 gm. on January 22; after that the weight increased again to 1560 gm. on February 10. Auscultation was negative on January 9 but on January 14 the first heart sound seemed defective and a diagnosis of mitral stenosis was

* Photomicrographs of this healing mitral lesion, as well as pictures of other healed and partially healed vegetations, were shown in the Scientific Exhibit at the meeting of The American Medical Association, Atlantic City, N. J., June 8 to 12, 1942. Program reference: Mac Neal, W. J., Spence, M. J., and Allen, A. C. Comparative study of the lesions of clinical and experimental endocarditis. Program of Scientific Assembly, p. 245, space 325; abstract in *J. A. M. A.*, 1942, **119**, 71.

recorded. On January 15 electrocardiographic and stethographic records were made and these were repeated on January 19. Problematic departures from the normal graphs were observed. Subsequently, auscultation of the heart was recorded as negative on February 3 and again on February 10. At this time the animal appeared healthy and had obviously recovered from infection of the blood stream. It was sacrificed on February 10, on the 47th day after initial inoculation.

Because of the clinical evidence of recovery and also on account of the anatomical condition observed after death, the treatment of the animal is worthy of detailed presentation. Therapeutic measures were instituted on January 1, 1943, 2 days after inoculation with streptococci had been discontinued. A specific bacteriophage, prepared in our laboratory, was injected intravenously in a dose of 10 cc. on January 1 at 9:00 A.M. and again at 1:00 P.M.; on January 2 at 9:00 A.M., 4:15 P.M. and 8:30 P.M.; on January 3 at 8:10 A.M., 4:10 P.M. and 12:00 midnight. These injections were continued with variations in time intervals until February 9. In addition, neoarsphenamine was administered intravenously in several courses as shown in the tabulated protocol of treatment (Table I). In brief, specific bacteriophage was given intravenously two or three times a day up to February 1 and once a day thereafter; neoarsphenamine was given several times a day in four courses: January 2 to 7, January 11 to 13, January 17 to 19, and January 24 to 26. The doses were spaced throughout the entire 24 hours in the earlier period. We do not wish to emphasize the significance of this particular therapeutic program and the detail is set down as a matter of record. It was planned to resemble the schedule of Osgood.[3]

Rabbit 133 was exsanguinated, under ether anesthesia, by bleeding from the carotid artery, followed by perfusion with saline solution and finally Helly's formalized Zenker's solution for immediate fixation of the tissues in the distended state. This method of fixation removed most of the blood from the vascular system and prevented post-mortem lysis of tissue elements and bacteria. Culture of the carotid blood remained sterile. Gross examination at necropsy revealed numerous irregular thickenings and nodules along the free margins of the mitral leaflets and moderate thickening of the aortic and pulmonary valves; the tricuspid appeared normal. The liver contained a few lesions of coccidiosis. Lungs, spleen, kidneys and brain were negative. A gross photograph of this heart is not available. Its general appearance was essentially similar to that of several other hearts in this group of animals, one of which is shown in Figure 13 (rabbit 392, series 5B).

On microscopical examination the myocardium of rabbit 133 ap-

TABLE I

Rabbit 133, Series 2B; Therapy from January 1 to February 9, 1943

All medication was given by intravenous injection. Clock time is shown on the basis of 24 hours starting with zero at midnight.

	Bacteriophage, 10 cc. doses at clock time below					Neoarsphenamine Amount and clock time below
Jan. 1		9:00	13:00			
2		9:00	13:00	16:15	20:30	0.8 mg. at 9:00, 13:00, 16:15, 20:30
3		8:10		16:10	24:00	0.8 mg. at 8:10, 16:10, 24:00
4		8:10		16:10		0.8 mg. at 8:10, 16:10
5	0:10	8:30		16:30		1.8 mg. at 0:10, 8:30; 2.0 mg. at 16:30
6	0:50	8:05		16:10		2.0 mg. at 0:50, 8:05, 16:10
7	0:10	8:05		16:05		2.0 mg. at 0:10, 8:05, 16:05
8	0:05	8:25		14:25		2.0 mg. at 0:05
9		9:00		15:00		
10		11:00		15:00		
11		9:00	12:30	16:00	20:10	2.0 mg. at 9:00, 12:30, 16:00, 20:10
12		8:30		16:00	23:55	2.0 mg. at 8:30, 16:00, 23:55
13		8:30		16:10	23:55	2.0 mg. at 8:30, 16:10, 23:55
14		8:30		16:10		
15		9:00		15:30		
16		10:00		14:13		
17		8:30	12:00	16:00	19:45	1.8 mg. at 8:30, 12:00, 16:00, 19:45
18		8:30		16:00	24:00	1.8 mg. at 8:30, 16:00, 24:00
19		8:30		16:00	23:40	1.8 mg. at 8:30, 16:00, 23:40
20		9:00		16:00		
21		9:00		16:00		
22		9:00		17:00		
23		10:00		14:30		
24		8:00		16:40		1.8 mg. at 8:00, 16:30
25		9:30		16:30		1.8 mg. at 9:30, 16:30
26		9:30		16:30		1.8 mg. at 9:30, 16:30
27		9:30		17:30		
28		11:00		15:30		
29		9:00		19:00		
30		9:00		16:30		
31		10:00		16:00		
Feb. 1		10:00		18:00		
2		10:30				
3		10:30				
4				14:00		
5		11:00				
6		11:00				
7		9:00				
8		9:00				
9		10:00				

peared negative. The sections through the mitral leaflets are of special interest. The irregular thickening and deformity of the valves is shown in Figures 14, 15 and 17. Bacteria were not found. The nodules were made up of fibroblastic cells and fibrils, and were covered by endothelium. Well defined mitotic division figures were readily found in the fibroblasts and also in the endothelial cells. Wandering cells were present in the stroma in very small numbers, only a lymphocyte here and there, and there was a suggestion of slight edema in the leaflets. A section through the aortic valve is shown in Figure 16 and the prominent nodule on the leaflet is represented in the colored drawing, Figure 18. Here also there are mitoses in the fibroblasts. Evidently there was at the time of death an active reparative process in the aortic and mitral leaflets, which we interpret as healing of previously infected vegetations at these sites.

The sections of liver and of kidney showed no bacterial lesions. The spleen was well distended by the perfusion. Its pulp spaces were wide in the sections and the follicles relatively small. In the lungs there were scattered small foci of cellular infiltration about some smaller arterioles but in general the lungs were normal.

These observations on rabbit 133 may be open to various interpretations. We are convinced, however, that this animal did have bacterial vegetations on the mitral and aortic valves, evidenced by bacteremia persisting until January 11, 12 days after inoculations had been discontinued, and by abnormal heart sounds on January 14, 15 and 19, as well as by the anatomical deformity and active process of repair in the mitral and aortic valves after death of the animal. If this be accepted, then the negative blood cultures after January 11, taken on January 18 and 25, and on February 3 and 9, together with the healthy appearance of the animal and the progressive increase in weight after January 22, as well as the structural changes in the heart after the animal was killed on February 10, may be properly interpreted as proof of arrest of the disease and healing of the local lesions in the heart.

Rabbit 362, series 8B, inoculated with Streptococcus viridans (salivarius), strain F 330-7, daily for 23 days; treated with penicillin and thiobismol; death after 23 days; healed mitral endocarditis with newly formed capillaries and hematogenous pigment in the substance of the valve.

Rabbit 362, series 8B, was inoculated with *Streptococcus salivarius*, strain F 330-7, isolated from the blood of a patient with bacterial endocarditis and passaged in a rabbit with production of cardiac vegetations. The intravenous inoculations were started on August 12 and continued daily through September 3 in doses of 0.125 to 0.375 cc. of

bacterial suspension. The rabbit weighed 2300 gm. on July 29, and 2165 gm. on August 10. Cultures of the blood taken on August 18 and 25 and on September 1 were positive. The one taken on September 3 remained negative, in spite of intravenous injection of bacteria on the previous day. Auscultation of the heart, daily except for six scattered omissions, was negative throughout. On September 2 severe diarrhea

TABLE II

Rabbit 362, Series 8B; Therapy from August 12 to September 2, 1943

Penicillin was administered by intramuscular, and thiobismol by intravenous, injection. Clock time is shown on the basis of 24 hours starting with zero at midnight.

	Penicillin, doses of 20 units each on the hour at clock time below								Thiobismol, doses of 2 mg. each on the hour at clock time below	
Aug. 12				10	13	16	19	22	10	16
13	1	4	7	10	13	16	19	22	10	16
14	1	4	7	10	13	–	–	22	10	–
15	1	4	7	10	13	–	–	–	–	–
16	–	–	–	10	13	16	19	22	10	16
17	1	4	7	10	13	16	19	22	10	16
18	1	4	7	10	13	16	19	22	10	16
19	1	4	7	10	13	16	19	22	10	16
20	1	4	7	10	13	16	19	22	10	16
21	1	4	7	10	13	–	–	22	10	–
22	1	4	7	–	13	–	–	–	–	–
23	–	–	–	10	13	16	19	22	10	16
24	1	4	7	10	13	16	19	22	10	16
25	1	4	7	10	13	16	19	22	10	16
26	1	4	7	10	13	16	19	22	10	16
27	1	4	7	10	13	16	–	22	10	16
28	1	4	7	10	13	–	–	22	10	–
29	1	4	7	–	13	16	–	–	–	–
30	–	–	–	10	13	16	19	22	10	16
31	1	4	7	10	13	16	19	22	10	16
Sept. 1	1	4	7	10	13	16	19	22	10	16
2	1	4	7	10	13	16	19	22	10	16
3	1			Total 2820 units in 22 days					Total 70 mg. in 22 days	

appeared and the rabbit died at 1:37 A.M. on September 3. The body was immediately fixed by arterial perfusion.

Treatment included penicillin in intramuscular doses of approximately 20 Oxford units at intervals of 3 hours, together with thiobismol in intravenous doses of 2 mg. given twice daily, with intermissions from time to time. The actual schedule of doses is shown in Table II.

The doses of penicillin were quite small, approximately 160 units per day for a rabbit of 2300 gm., or about 70 units per kg. per day. The

dose of thiobismol was relatively larger, about 1.7 per kg. daily, approaching the upper limit of continuous doses for a human patient.

At necropsy the mitral leaflets were rough and thickened and there were distinct nodules on the posterior flap. The tricuspid leaflets appeared very slightly thickened. The aortic cusps were definitely stiffer than normal. The lungs were congested and there was extravasation of blood in the right lung. A few lesions of coccidiosis were seen in the liver. The spleen was somewhat enlarged, 55 by 10 by 4 mm., and the kidneys were grossly normal.

Microscopical study of the mitral valve revealed irregular angulation and distortion together with nodular fibrous thickenings attaining a diameter of 1 mm. The fibrils and cells of the valve were somewhat irregular in their arrangement and a few polynuclear leukocytes and lymphocytes were included in the stroma. Adhesions between adjacent folds of the leaflets had resulted in the formation of included vascular spaces lined by endothelium and containing blood. Continuous with these spaces there were narrower channels presenting the appearance of venous capillaries filled with blood. Apparently these new vessels communicated with the cavity of the heart and not with the coronary circulation. Throughout the thickness of the mitral leaflet there were clumps of yellowish brown pigment, much of it contained within phagocytic cells. This pigment was evidently derived from erythrocytes which had entered the stroma through defects in the endothelial covering of the valve during the period of active inflammation.

A portion of deformed mitral leaflet is depicted in Figure 19 and in greater detail in the colored drawing, Figure 21. The granules of brown pigment, chiefly contained within phagocytic cells in the interior of the structure, and a newly formed vascular space within the valve can be distinguished. A healed vegetation on the pulmonary valve of this animal is shown in Figure 20.

Rabbit 370, series 9B, inoculated with Streptococcus viridans (salivarius), strain F 345-8, daily for 18 days; early systolic murmur; treatment with penicillin; death on the 22nd day; healed mitral and aortic endocarditis; healed pulmonary endarteritis.

Rabbit 370, series 9B, weighed 1625 gm. on October 4. Auscultation of the heart was negative on October 7 and 11. *Streptococcus salivarius*, strain F 345-8, was given intravenously in doses of 0.125 to 0.250 cc. of bacterial suspension daily on October 10 to 27, inclusive. The temperature was 102.0° F. on October 9 and rose on October 11 to 107° in the morning and 106.8° in the afternoon. More remarkable was the appearance of a loud systolic blowing murmur first heard on the morning of October 12. A diagnosis of early mitral vegetative

endocarditis was confidently accepted. The murmur was much the same on October 13 but on October 14 the heart sounds were weak and the murmur was not recognized. Weak sounds were noted on October 16 and 20 but the murmur was not again detected. Cultures of the blood taken on October 12, 13 and 17 gave positive growth; those taken on October 24 and 27 remained negative. Until October 24 the animal's temperature was irregular and frequently reached high

TABLE III

Rabbit 370, Series 9B

Treatment with penicillin by intravenous (V) or intramuscular (M) injections. Clock time is shown on the basis of 24 hours, starting with zero at midnight. Doses are expressed in hundreds of (Oxford) units of penicillin.

Hour	0	2	4	6	8	10	12	14	16	18	20	22
Oct. 12											10-V	5-V
13	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V
14	5-V	5-V	5-V	5-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
15	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
16	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-M
17	20-M	20-M	20-M	20-M	20-V	20-V	20-M	20-V	20-V	20-V	20-V	20-V
18	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-M	20-V	20-V
19	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	-
20	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
21	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
22	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
23	20-M	20-M	20-M	20-M	20-M	20-M	20-V	20-V	20-V	20-V	20-V	20-V
24	20-M	-	-	-	20-M	20-M	20-M	20-V	20-M	20-M	20-M	20-M
25	20-M	20-M	20-M	20-M	20-M	20-V	20-M	20-M	20-M	20-M	20-M	20-M
26	20-M	20-M	20-M	20-M	20-M	20-V	20-M	20-M	20-M	20-M	20-M	20-M
27	20-M	20-M	20-M	20-M	20-M	10-V	10-M	10-M	10-M	10-M	10-M	10-M
28	10-M	10-M	10-M	10-M			Died at 7:30 A.M., October 28					

levels; *i.e.*, 107.2,° 106.4,° 106.0,° 105.8,° 105.6,° 105.0,° but after October 24 the range was 101.2° to 104.0.° The weight of the rabbit diminished from the original 1625 gm. to 1615 gm. on October 9, 1585 gm. on October 15, 1440 gm. on October 23 and 1200 gm. on October 27. Death took place at 7:30 A.M. on October 28 and the body was at once fixed by arterial perfusion.

Treatment with penicillin alone was initiated on October 12 in an attempt to overcome the clearly evident early mitral endocarditis by a thorough course of penicillin in large doses, administered by intravenous or intramuscular injection as shown in Table III. The amount of penicillin used in the 17 days was 282,500 Oxford units, of which 176,500 were given intravenously and 106,000 by intramuscular injection. As the animal weighed 1625 gm., the penicillin used would corre-

spond to about 8.5 million units for a person weighing 50 kg., apparently an excessive amount.

At necropsy the posterior leaflet of the mitral valve appeared slightly thickened and the other valves were regarded as grossly normal. There were scattered lesions of coccidiosis in the liver. The spleen measured only 30 by 5 by 2 mm. before perfusion and 40 by 7 by 2 mm. after perfusion. The lungs and kidneys were grossly normal.

Microscopical sections through the mitral leaflets revealed a very marked thickening near the free margin and adjacent to the attachments of the tendinous cords. The deformity evidently had resulted from an inflammatory lesion but the precise character and extent of this lesion were no longer recognizable. In the substance of the leaflets there were very few wandering cells and only occasional red blood cells. Healing had evidently been complete for some time. The aortic leaflets also were irregularly thickened. In the lungs of this animal there were nodular thickenings in the walls of the smaller pulmonary arteries in which multinucleated giant cells and clefts indicating cholesterol deposits were present. The healed state of these pulmonary arterial lesions corresponded to the healed state of the cardiac valves.

A section through the deformed mitral leaflet is illustrated in Figure 22 and a section of the aortic valve in Figure 23.

Discussion and Comment

From observations on the course of experimental endocarditis lenta in rabbits and the study of the tissues after death, we have gained certain ideas in regard to the healing processes and the possible arrest of the disease in local lesions and in the entire animal. These observations seem to throw light on the course of events in the human disease and to promote a better understanding of the difficulties which confront the physician in his attempt to bring about an arrest of bacterial endocarditis and healing of the local lesions in his patient, and to some extent point the way to overcome these difficulties.

Relief of local stress at the site of the lesions is a primary indication and this may be favored by strict rest in bed and promotion of emotional calm. Intelligent, sympathetic nursing and freedom from annoyance by visitors and by other patients are desirable. General measures to maintain bodily resistance at a high level are of value. However, it must be evident that these measures will usually prove to be inadequate, once the vegetations with their bacterial colonies in necrotic fibrinous deposits have become well established. An increase of antibacterial agents in the blood is greatly to be desired. This increased concentration of these agents should be maintained for a pro-

longed period so as to restrain the multiplication of the streptococci, hamper the elaboration of their poisons and thus permit more effective eradication by bacteriolytic ferments and the intimate approach of phagocytic cells to engulf and digest them. It must also permit the successful proliferation of fibroblastic and endothelial cells in and on the fibrinous deposits to bring about the encapsulation of the infected material and its eventual organization into sterile scar tissue. Fortunately, there exist antibacterial agents which are known to exhibit a restraint upon the growth of the streptococci in the culture tube. These are the agents which we have attempted to select for experimental test in the diseased rabbits. Anti-infectious agents of promising potency in the culture tube may be expected, in many instances, to fail in the living animal because the conditions in the cardiac vegetation may be less favorable to their action and because the anti-infectious agents themselves may exert deleterious influences upon the tissues of the animal. The experimental therapeutic tests in the animals, therefore, may supply information to supplement the results of observations in the culture tube and thus aid in the selection of those anti-infectious agents most potent in the animal body and relatively free from deleterious effects, and these would appear to offer greatest promise for clinical trial in the human patient. The animal experiments may also aid in determining the most promising detailed program of therapeutic application of these agents, a matter of some importance.

In this communication we have attempted to present some of the results obtained in a prolonged study, in which many difficulties have been encountered. We are convinced that local healing of infected valvular vegetations and complete arrest of the disease process can be achieved in experimental endocarditis lenta of the rabbit. The therapeutic agents of greatest value include, among the synthetic chemicals, thiobismol, neoarsphenamine, mapharsen, sulfadiazine and sulfapyridine and, in the biological group, penicillin and bacteriophages. In our experiments the favorable effect of penicillin in experimental endocarditis due to *Streptococcus salivarius* has been conspicuous. In the experimental disease produced by inoculation with *Streptococcus faecalis* (enterococcus) the specific bacteriophages, in combination with thiobismol or neoarsphenamine, have seemed more promising. The chemotherapeutic agents alone have been less effective but we believe that they may exert a favorable influence.

The experimental therapy has, of course, already been extended to human patients by others [4-7] as well as by ourselves.[8-11] Here, as in the rabbit, we believe that the prolonged maintenance of a mild bacteriostatic concentration of the antibacterial agents in the circulating blood,

so as to restrain active bacterial growth at the site of the lesions, is the important desideratum. This favors local healing and at the same time tends to protect the distant organs from embolic insults. Complete arrest of the disease is in some respects similar to arrest in pulmonary tuberculosis. One cannot appraise with certainty the exact state of the completely or partially healed internal lesions. Prognosis in the arrested stage should, therefore, be guarded.

Summary

1. Defense against experimental infection with streptococci of the viridans group brings into play a variety of mechanisms.

2. In the initial stage of bacteremic invasion the microbes are abundantly phagocytosed by the wandering leukocytes and especially by the fixed endothelial cells of the smaller blood vessels in general and to a less extent by the endocardial endothelium, and they are largely exterminated by digestion within these phagocytes.

3. After a bacterial vegetation has become established on a valve of the heart, the streptococci thrown off from it into the blood stream are largely disposed of by a similar process of phagocytosis. However, the bacterial colonies in the fibrinous vegetation, by their active growth and elaboration of toxic substances, ordinarily keep the host cells at a distance and continue to flourish.

4. To conquer these local bacterial nests, the first requirement is restraint of their active growth, which is not readily accomplished by the unaided defense mechanisms of the host. Second in importance appears to be the deposit of bacteria-free fibrin over the surface of the infected lesion, to serve as a framework for leukocytes, fibroblasts and endothelial cells, which come in to ingest the bacteria and organize the scar.

5. Restraint of bacterial proliferation is exercised not alone by antibacterial substances in the normal blood plasma and substances produced by the bacteria themselves, but may evidently be favored in a significant manner by chemical and biological therapeutic agents introduced into the body from without.

6. Local healing and complete general arrest of the experimental disease have been sometimes accomplished in rabbits and there is good reason to believe that similar success may be attained in the human disease.

REFERENCES

1. Mac Neal, W. J., Spence, M. J., and Slavkin, A. E. Early lesions of experimental endocarditis lenta. *Am. J. Path.*, 1943, **19**, 735–749.
2. Mac Neal, W. J., Spence, M. J., and Slavkin, A. E. Progressive experimental endocarditis lenta. *Am. J. Path.*, 1944, **20**, 95–119.

3. Osgood, E. E. Neoarsphenamine therapy of bacterial infections, with a method of administration to maintain uniform blood levels for the treatment of serious staphylococcic infections and subacute bacterial endocarditis. *Arch. Int. Med.*, 1942, **69**, 746–765.
4. Florey, M. E., and Florey, H. W. General and local administration of penicillin. *Lancet*, 1943, **1**, 387–397.
5. Keefer, C. S., Blake, F. G., Marshall, E. K., Jr., Lockwood, J. S., and Wood, W. B., Jr. Penicillin in the treatment of infections. *J. A. M. A.*, 1943, **122**, 1217–1224.
6. Loewe, L., Rosenblatt, P., Greene, H. J., and Russell, M. Combined penicillin and heparin therapy of subacute bacterial endocarditis. Report of seven consecutive successfully treated patients. *J. A. M. A.*, 1944, **124**, 144–149.
7. Loewe, L., Rosenblatt, R., Greene, H., and Russell, M. Combined penicillin and heparin treatment of subacute bacterial endocarditis; experimental and clinical study. Presented before the New York Section, *Soc. Exper. Biol. & Med.*, Feb. 16, 1944.
8. Mac Neal, W. J., and Poindexter, C. A. Arrest of endocarditis by penicillin. Presented before the New York Heart Association, Feb. 1, 1944.
9. Mac Neal, W. J., Blevins, A., Slavkin, A. E., and Poindexter, C. A. Arrest of endocarditis due to *Streptococcus viridans*. Presented before the New York Section, *Soc. Exper. Biol. & Med.*, Feb. 16, 1944.
10. Mac Neal, W. J., Blevins, A., and Poindexter, C. A. Clinical arrest of bacterial endocarditis by bacteriostatic agents, particularly penicillin. Presented before the Clinical Research Meeting, New York Academy of Medicine, April 5, 1944. Abstract in *Bull. New York Acad. Med.*, 1944, **20**, 415–416.
11. Mac Neal, W. J., Blevins, A., and Poindexter, C. A. Clinical arrest of endocarditis lenta by penicillin. *Am. Heart J.*, 1944, **28**, 669–679.

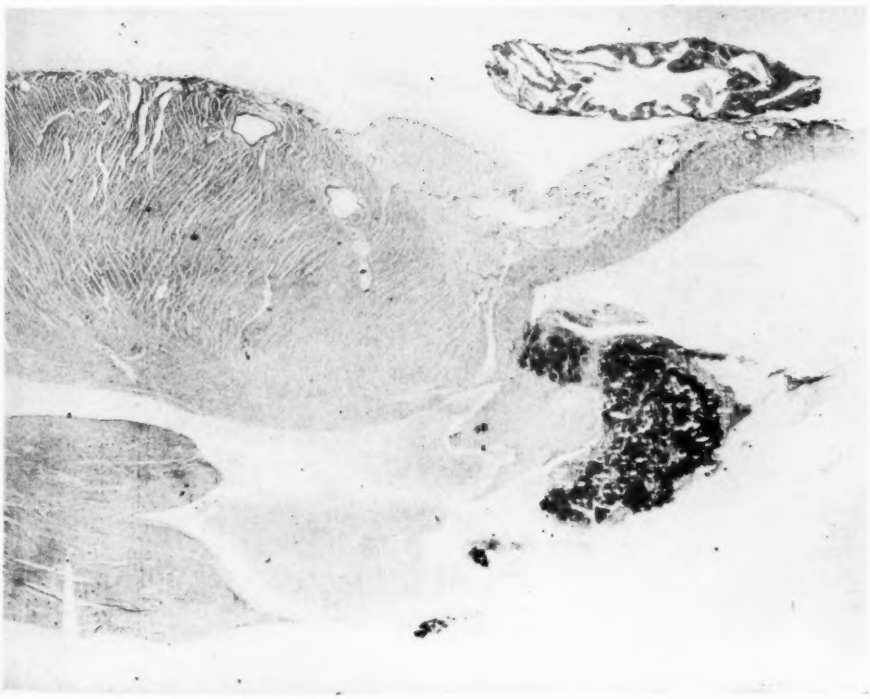
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Photograph of the left side of the opened heart of rabbit 166, series 4B, which died on the 30th day after initial inoculation with *Streptococcus faecalis* and subsequent treatment with thiobismol and specific bacteriophage. Part of the large, ragged mitral vegetation is shown.

FIG. 2. Photomicrograph at low magnification of a section through the mitral vegetation of rabbit 166, series 4B, stained by the method of Brown and Brenn. The mass of fibrin containing bacterial colonies, wandering cells and many irregular spaces rests chiefly on the auricular surface of the much thickened and distorted mitral leaflet. The very dark spots near the surface of the vegetation and many of the darker gray spots in the interior represent colonies of streptococci. There is also considerable cellular exudate in and near the base of the swollen leaflet and also in parts of the fibrinous vegetation itself. The thick ventricular wall and adjacent papillary muscle are easily recognized for orientation.



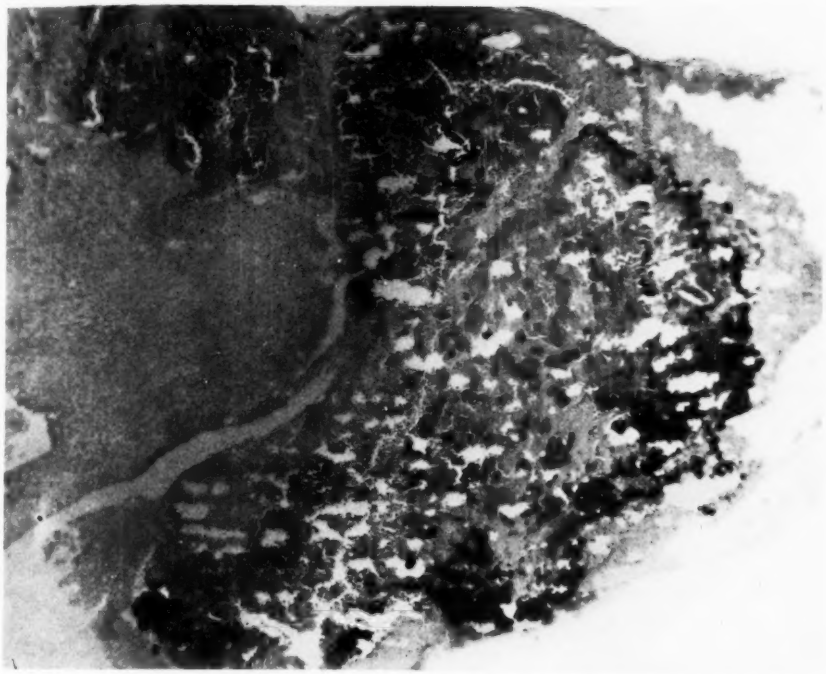
Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

PLATE 42

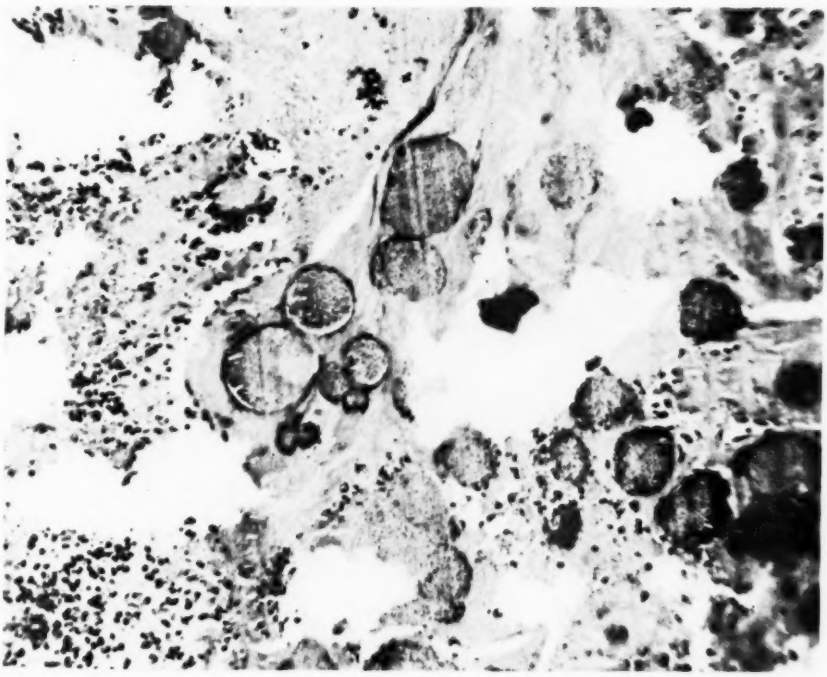
FIG. 3. Photomicrograph showing more detail of the same vegetation. The bacterial colonies about the periphery retain a deep stain but they are for the most part covered over by a layer of fibrin, irregular in structure and thickness. The deeper bacterial colonies, on the other hand, show variable degrees of bacteriolysis, affecting especially the central portions of the colonies. At the midline of the figure and somewhat to the right of the center there is an irregular space containing at the left a dark-stained irregular clump of necrotic material. Below this cleft there are several bacterial colonies in which bacteriolysis is already far advanced. At the left near the lower corner is a large dark-stained mass of necrotic material and near this there is cellular exudate without well defined bacterial colonies. A small part of this exudate is represented in color in Figure 6.

FIG. 4. Photomicrograph of part of the mid-portion of the same vegetation. The irregular cleft with dark-stained necrotic clump is again recognizable to the left of the center of this figure. Above it, and to the left, are several bacterial colonies not in good focus, but showing nevertheless distinct central bacteriolysis. Left of the cleft is a group of three small bacterial colonies almost in contact with each other, which are shown in color in Figure 5. At their left is a colony largely destroyed by bacteriolysis and other large lytic colonies appear nearby. Below are smaller colonies, apparently confluent, with deeply stained cocci about the periphery. Cellular exudate is recognizable below and to the right but the bacteria present in this exudate cannot be distinctly seen at this magnification.

3



4

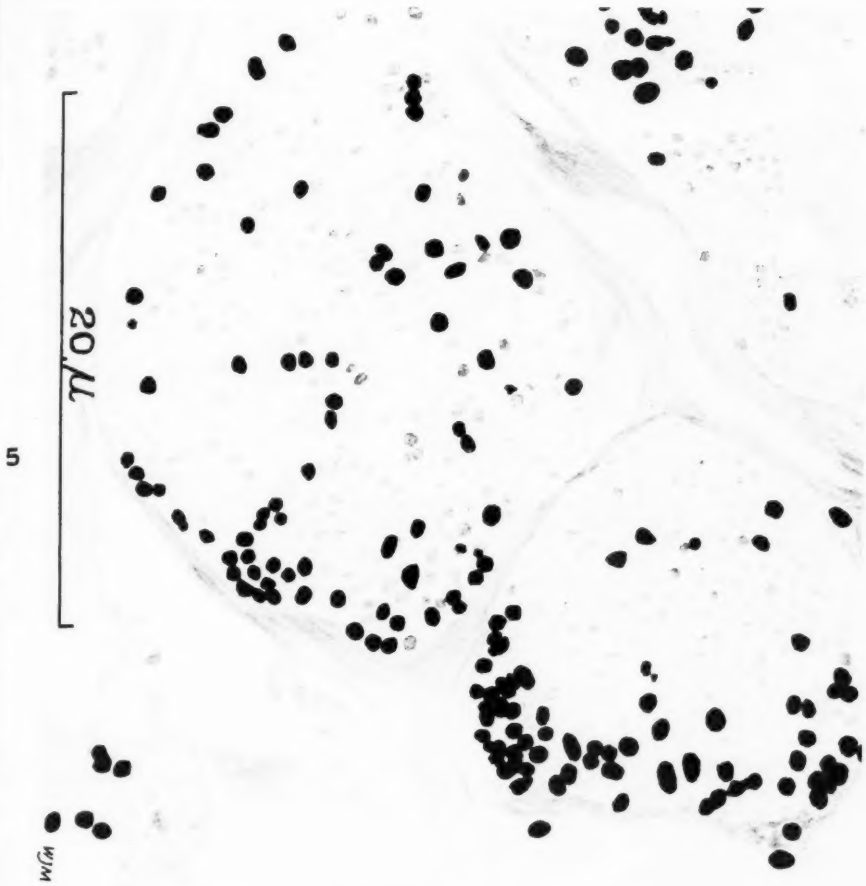


Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

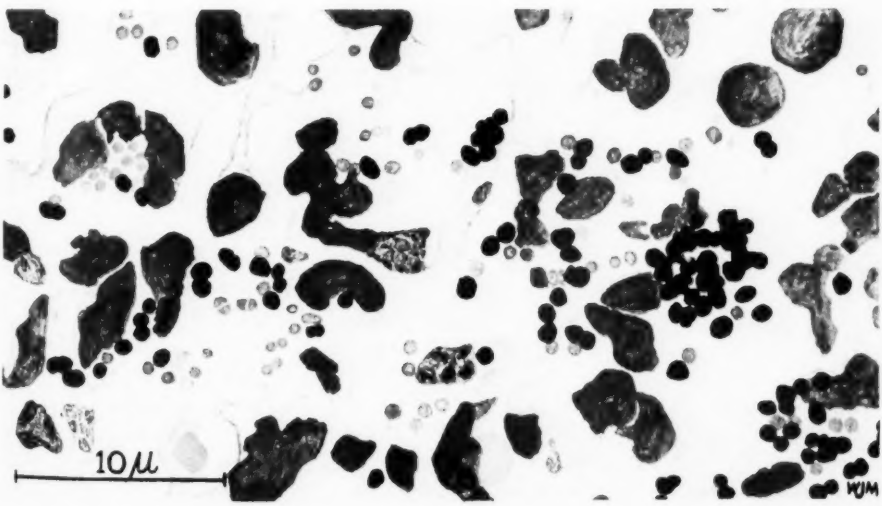
PLATE 43

FIG. 5. Drawing at high magnification of the three bacterial colonies mentioned in the legend for Figure 4. The various stages of bacteriolysis and fragmentation of the streptococci can be recognized. Some of the bacteria still take the stain very well. Not a single phagocytic cell is present in the field of this drawing, nor is there any host cell present.

FIG. 6. Drawing of a small part of the cellular exudate mentioned in the legend of Figure 3. The exudate is full of deformed wandering cells adherent to each other and partly fragmented. They contain numerous phagocytosed cocci in various stages of growth and bacteriolysis. The streptococci are also lying free in the fluid serum. Apparently some agent or agents in solution have acted to permit these wandering cells to approach intimately the abundant bacterial groups and to engulf them.



5



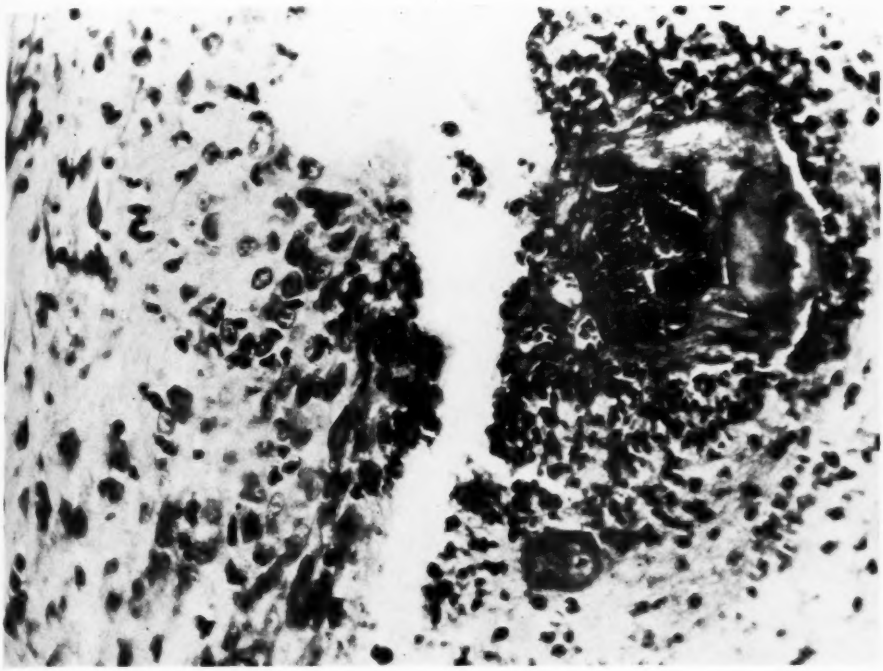
6

Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

PLATE 44

FIG. 7. Photomicrograph of a small portion of a section through this same mitral vegetation of rabbit 166, stained with hematoxylin and eosin. A bacterial colony surrounded by bacteria-free fibrin is walled in by a zone of wandering cells and these in turn are covered by loose clot containing red and white blood cells. The substance of the mitral leaflet, to the left, is edematous and invaded by wandering cells. There is also active proliferation of its elements, evidenced by the rather numerous division figures. The mother star of a fibroblast in mitosis is recognizable near the midline below the center.

FIG. 8. Photomicrograph of a section through part of a swollen mitral leaflet of rabbit 145, series 2B, stained by the method of Brown and Brenn. This rabbit died on the fifth day after initial intravenous inoculation. The black spot to the right and below the center represents a bacterial colony which is shown in greater detail in Figures 9 and 10.

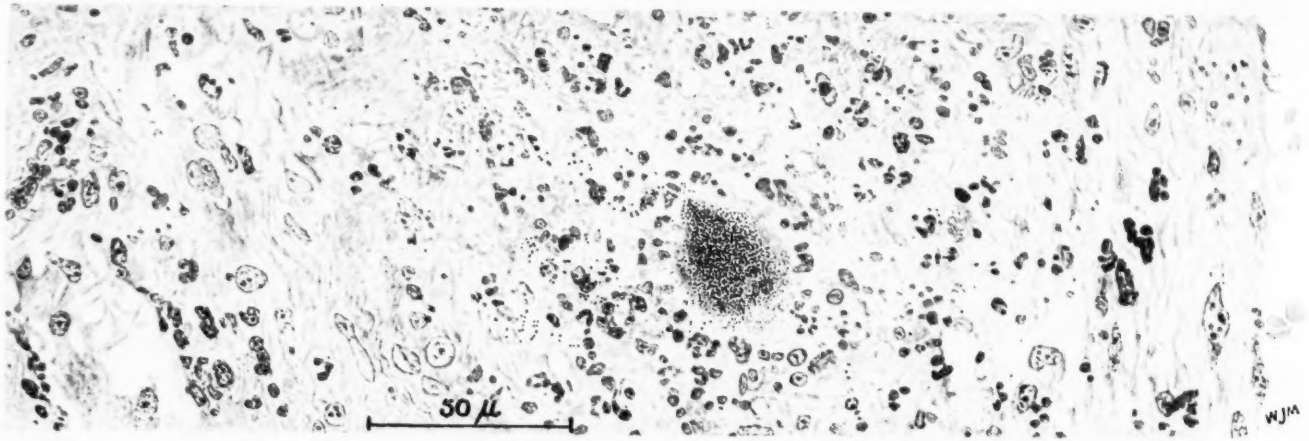


Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

PLATE 45

FIG. 9. Drawing of the bacterial colony of Figure 8 with adjacent substance of the leaflet extending to the endothelial surface at the right. There are many individual bacteria scattered in the stroma even at some distance from the compact colony.

FIG. 10. Drawing, at high magnification, of the lower margin of the bacterial colony of Figure 8, showing stages of bacteriolysis at the periphery of the colony and phagocytosis of some of the streptococci in the neighborhood.


50 μ
WJM

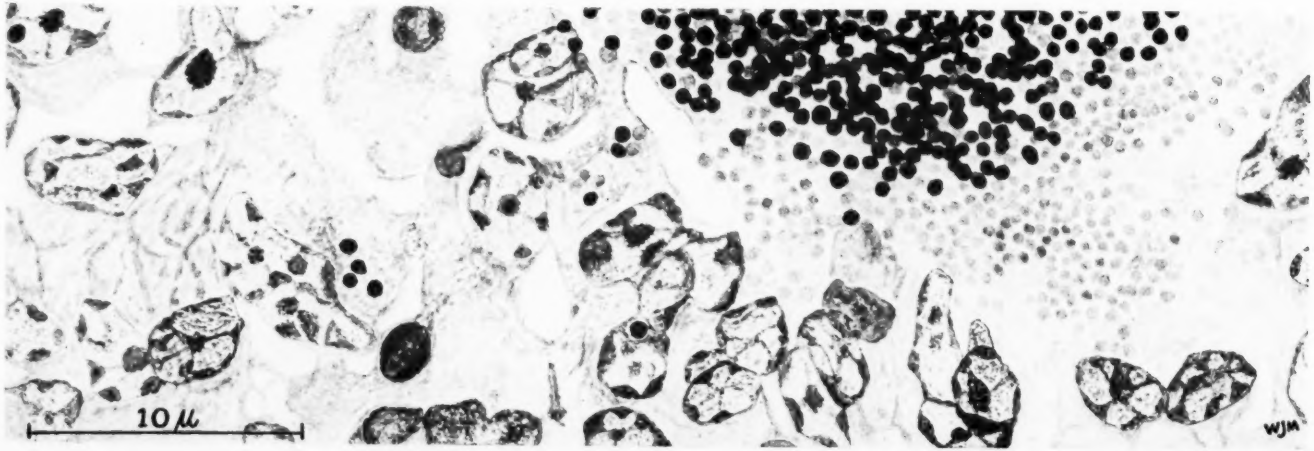
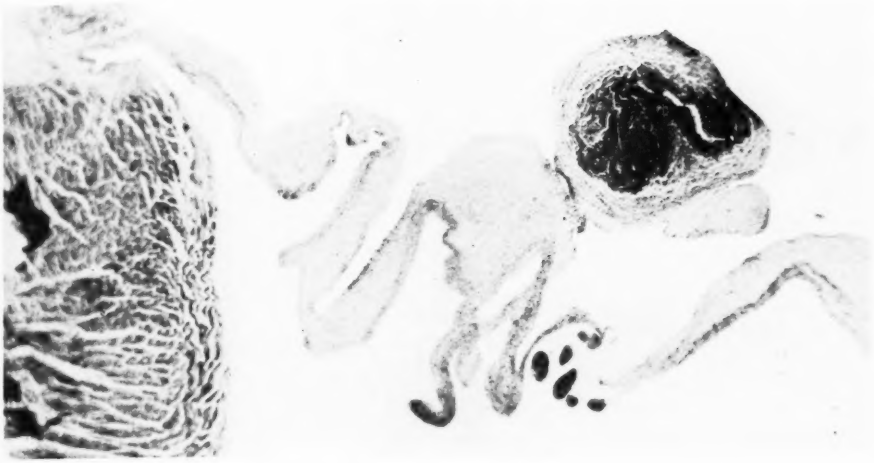
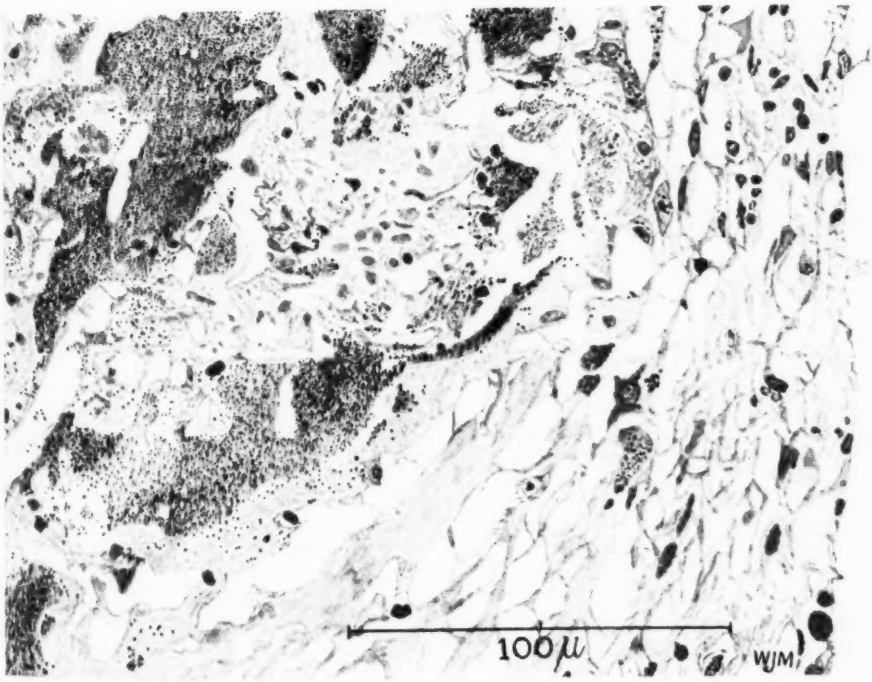

10 μ
WJM

PLATE 46

FIG. 11. Photomicrograph of a section of mitral leaflet of rabbit 593, series 14A, which died on the 18th day after initial intravenous inoculation, stained with hematoxylin and eosin. The vegetation is situated well out on the auricular face of the valve. There is an irregularly outlined center of fibrin, containing bacteria and wandering cells. Around this is a fairly complete capsule of fibroblastic tissue covered over with smooth endothelium.

FIG. 12. Drawing of a companion section of this same mitral leaflet of rabbit 593, stained by the method of Brown and Brenn, showing a small portion of the outer layers. The endothelial surface is at the right. The masses of bacteria lack the rounded contour of growing bacterial colonies and nucleated cells have been able to approach them and even to engulf many streptococci.





Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

PLATE 47

FIG. 13. Photograph of a gross section through the mitral ring of rabbit 392, series 5B, showing nodular deformity of the leaflets subsequent to intravenous inoculation with *Streptococcus viridans* and successful treatment with bacteriophage and thiobismol.

FIG. 14. Photomicrograph at low magnification of a section through the mitral ring of rabbit 133, series 2B, in which experimental endocarditis was evidently produced by intravenous inoculation and subsequently healed after treatment with bacteriophage and neoarsphenamine. The mitral leaflet is thickened and deformed.

Mac Neal, Blevins, Pacis and Slavkin

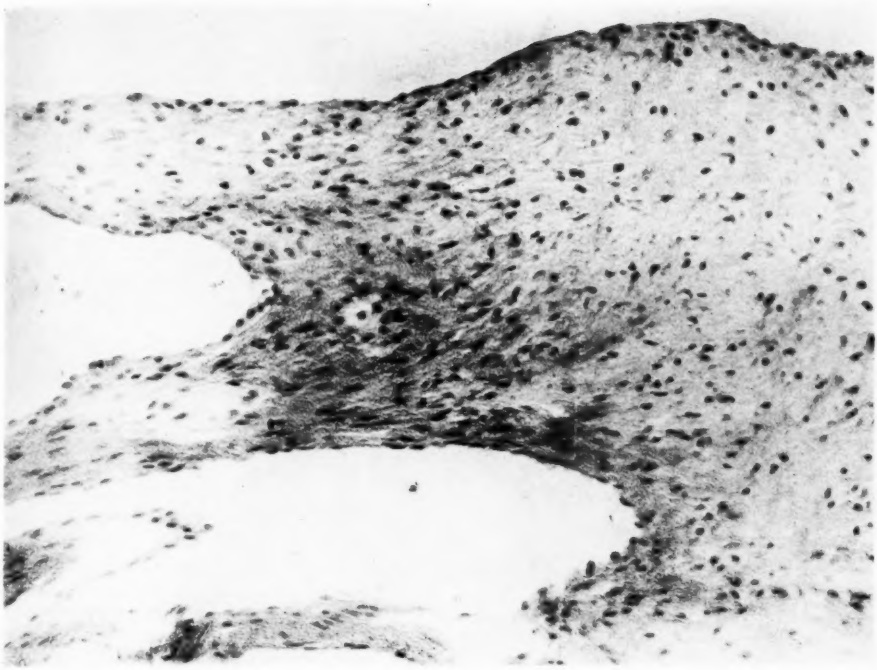
Experimental Endocarditis Lenta

PLATE 48

FIG 15. Photomicrograph of part of a companion section in this same series of sections through the mitral leaflet of rabbit 133, stained with hematoxylin and eosin, showing the irregular arrangement of cells and fibers in the thickened leaflet and, near the fork at the upper left of the figure, two diasters cut in a fortunate plane.

FIG. 16. Photomicrograph of an oblique section through the aortic ring of rabbit 133, series 2B, stained with hematoxylin and eosin. On the ventricular face of one of the leaflets there is an irregular fibrous projection shown in more detail in Figure 18.

15

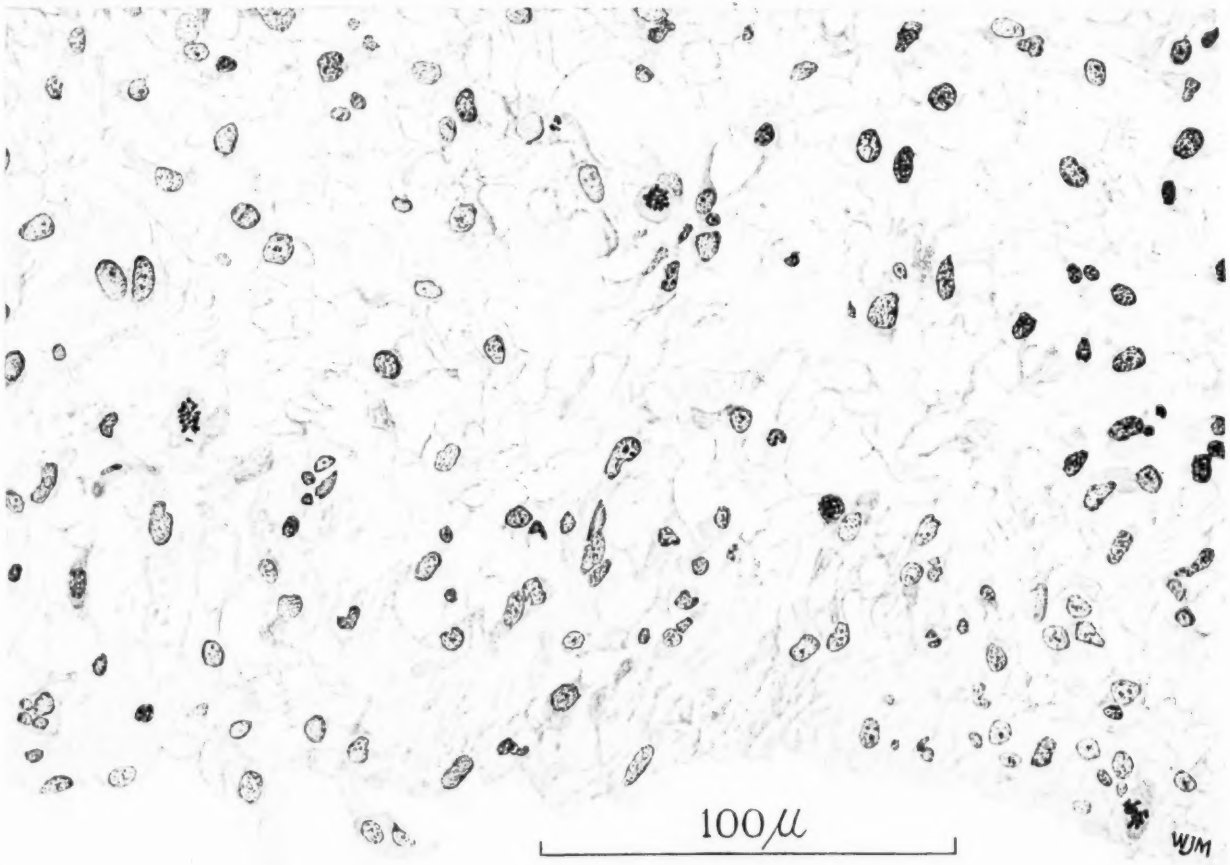


16

Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

PLATE 49

FIG. 17. Drawing of a small superficial portion of a section through a nodular thickening on the mitral leaflet shown in Figure 15, stained with hematoxylin and eosin. There is a mitotic figure in an endothelial cell at the lower right corner and two mitoses in fibroblasts to the left and toward the top of the drawing. The presence of a few lymphocytes and also the moderate edema suggest that extermination of the infectious agent may have been only recently accomplished.



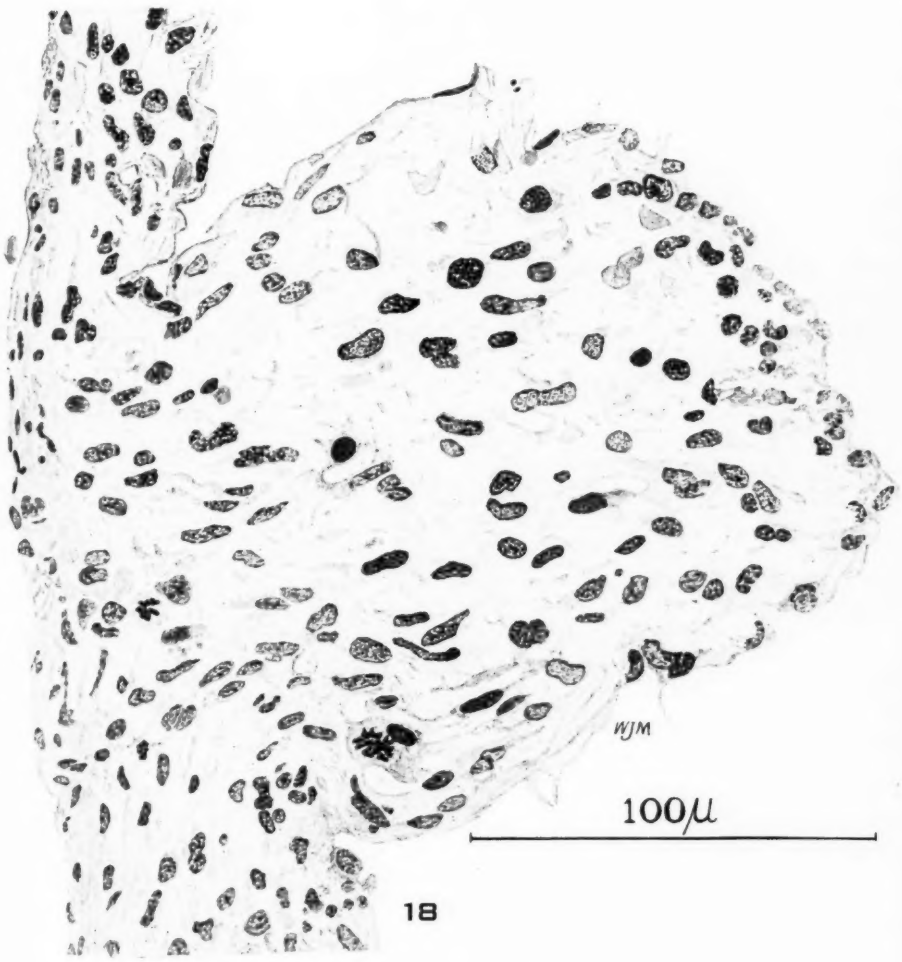
17

Mac Neal, Blevins, Pacis and Slavkin

Experimental Endocarditis Lenta

Plate 50

Fig. 18. Drawing of the nodular prominence on the aortic valve seen in Figure 16. The nuclei of the endothelial cells are irregular in size and shape. The nodule is made up of fibroblasts and fibers with spaces less prominent than in the mitral nodule of Figure 17. There are, however, nuclear division figures, a few lymphocytes and one erythrocyte included in this section of the nodule.

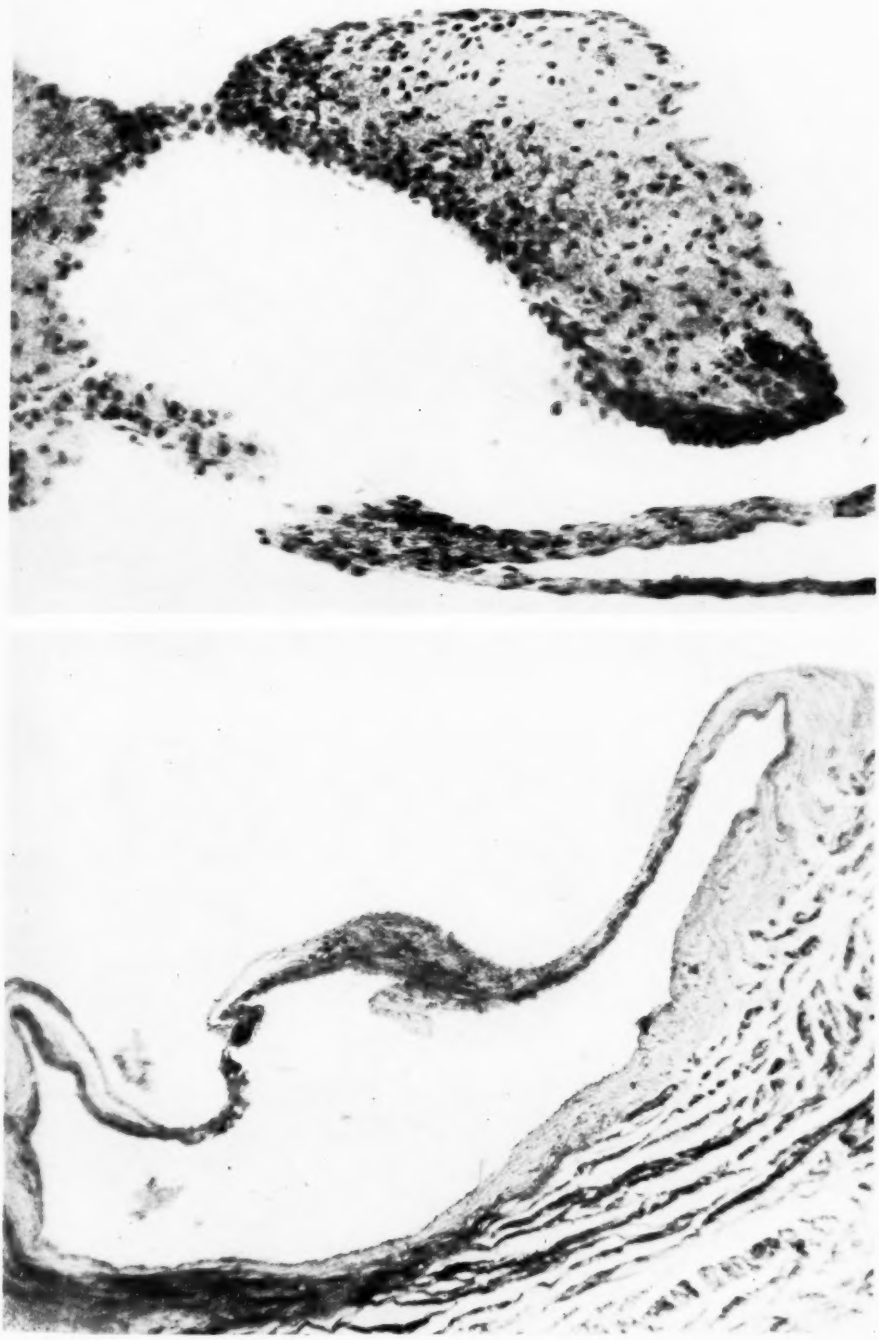


Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

PLATE 51

FIG. 19. Photomicrograph of a small portion of a section of the deformed mitral valve of rabbit 362, series 8B, stained with hematoxylin and eosin. This rabbit was inoculated intravenously with *Streptococcus salivarius* daily for 23 days and treated with penicillin and thiobismol. The endothelial cells are irregular in form, size and arrangement, and form several compact strata near the tip of the nodule, shown at the lower right. Above this region is an included vascular space or capillary, filled with blood. More detail is shown in Figure 21.

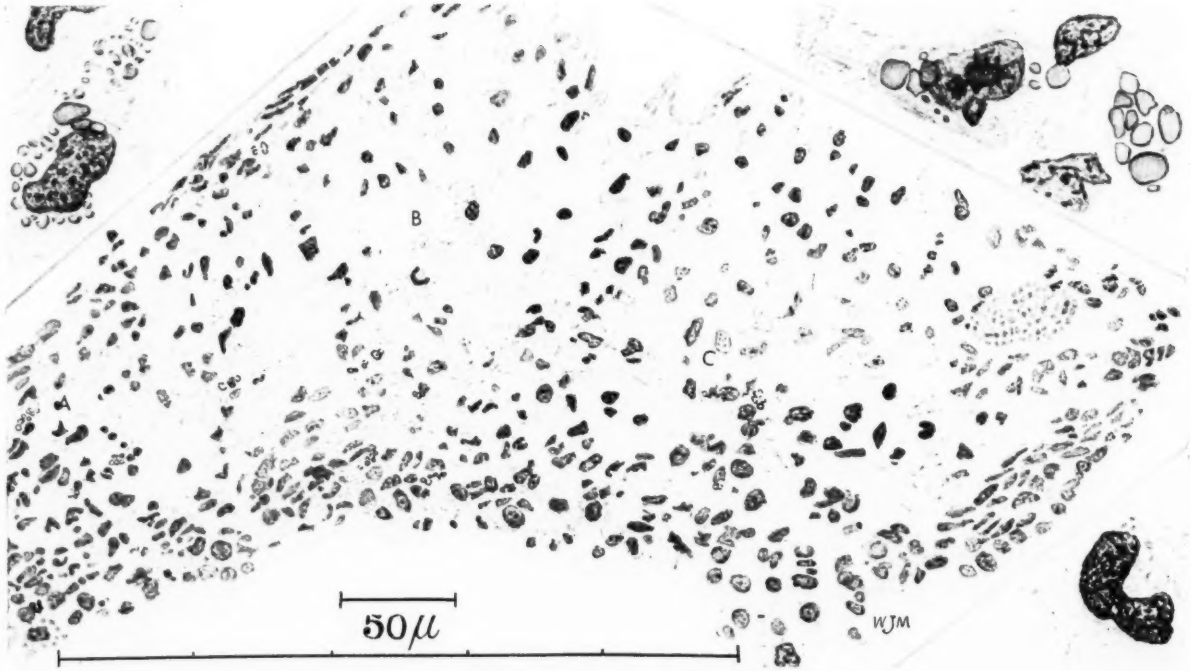
FIG. 20. Photomicrograph of a section stained with hematoxylin and eosin passing through a deformed pulmonary semilunar cusp of rabbit 362, series 8B. Apparently the irregular thickening represents a healed lesion of endocarditis on this valve.



Mac Neal, Blevins, Pacis and Slavkin Experimental Endocarditis Lenta

PLATE 52

FIG. 21. Drawing of part of the deformed mitral leaflet of Figure 19. A group of brown granules at the extreme left of the central figure is represented at higher magnification (see scale below) in the upper left corner. A similar collection of granules at the right center of the large figure is shown on the larger scale in the upper right insert. A cell at the left center of the central figure is depicted on the larger scale as an insert at the lower right corner.



21

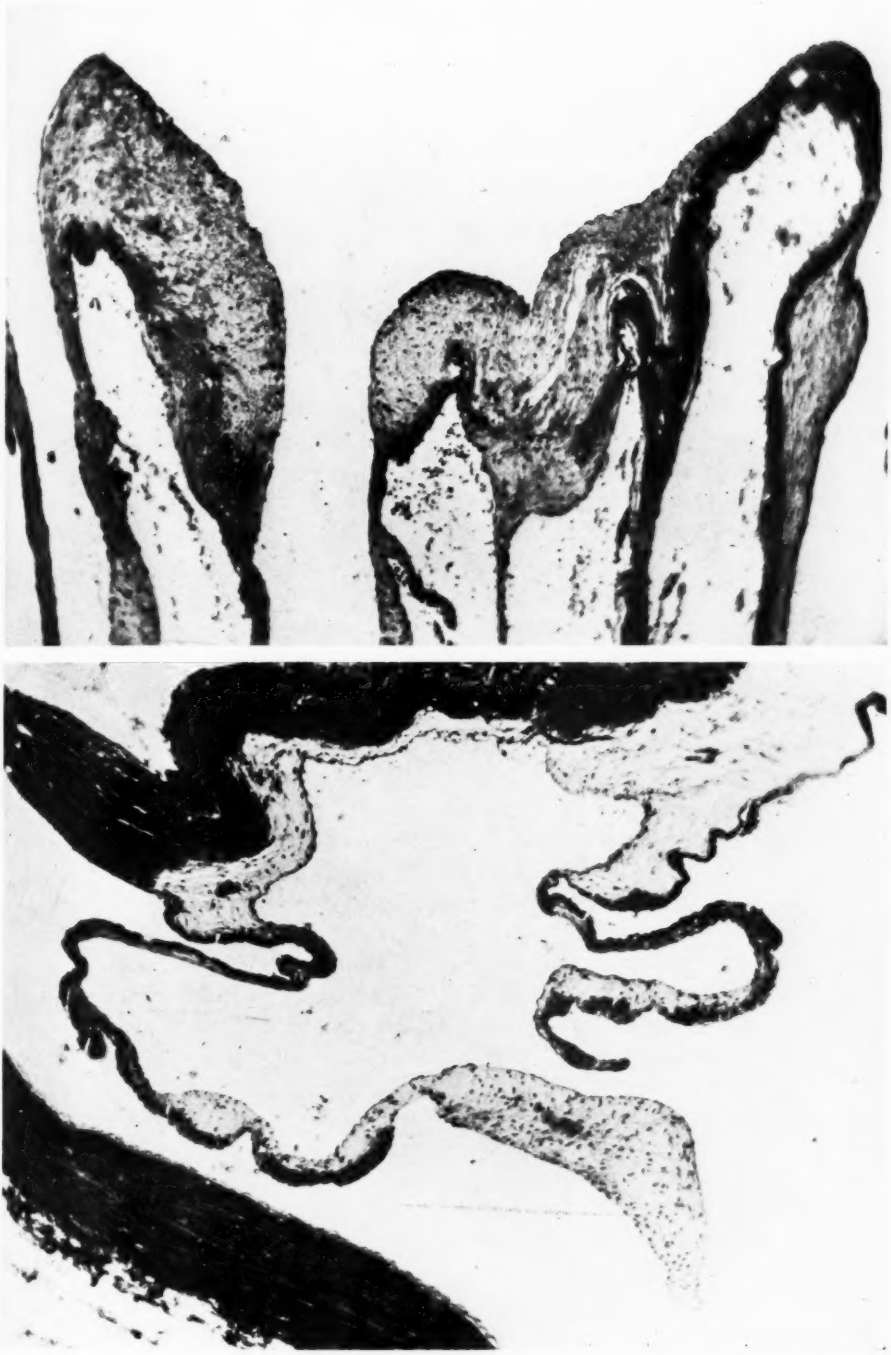
Mac Neal, Blevins, Pacis and Slavkin

Experimental Endocarditis Lenta

PLATE 53

FIG. 22. Photomicrograph of a section stained with hematoxylin and eosin, through part of the mitral valve of rabbit 370, series 9B. This rabbit was inoculated with *Streptococcus salivarius*, developed a loud mitral murmur and high fever and was treated with large doses of penicillin. The mitral leaflets are thickened and deformed.

FIG. 23. Photomicrograph of a section stained with hematoxylin and eosin, passing obliquely through the aortic ring of rabbit 370. There is irregular thickening of the aortic cusps indicating a healed lesion of bacterial infection.



Mac Neal, Blevins, Pacis and Slavkin — Experimental Endocarditis Lenta

STUDIES ON TUMORS OF THE TESTIS

II. The Morphology of Testicular Tumors of Dogs *

Charles Huggins, M.D., and Ricardo Pazos, Jr., M.D.
(From the Department of Surgery, the University of Chicago, Chicago, Ill.)

The testicular tumors of dogs are of special interest because of their frequency, the rarity of metastasis and often because of their production of androgen or estrogen. While several types of canine testicular tumors closely resemble the tumors of man, others do not have a counterpart in human pathology. This study is concerned with the morphology of 64 tumors of the testes of 41 dogs with special reference to the nontumorous testicular tissue, the prostate as an indicator of hormonal production and the extent of necrosis in the tumor.

Despite a complex terminology, most workers agree that the common testicular tumors in dogs may be classified into three groups: (1) interstitial cell tumors; (2) seminomas (embryonal carcinoma, grosszelligen Tumoren, seminal epithelioma); (3) tubular adenomas (adenocarcinoma, Sertoli cell tumors). Early reports of isolated cases of canine testicular tumors have been recently reviewed.[1,2] Kunze [3] observed 23 canine testicular tumors which were classified thus: 13 interstitial tumors, 5 seminomas, 4 adenomas, 1 spindle cell tumor. Pallaske [4] observed 38 tumors in the testes of 107 dogs; 31 were interstitial tumors and 7 were apparently derived from germinal epithelium. Peyron, Blanchard and Salomon [5] described 31 interstitial tumors and 5 seminomas in the testes of old dogs. An extensive series was reported by Schlotthauer, McDonald and Bollman [1] who studied 82 spontaneous tumors in 59 testes of 48 dogs without evident metastases; there were 51 interstitial cell tumors, 25 seminomas and 6 adenocarcinomas. The same group [6] analyzed the seminomas in detail—7 of them were diffuse while 18 seminomas were wholly or partly confined to the tubules; the latter observation supported the idea of Chevassu [7] that seminomas arise from epithelium of seminiferous tubules. Innes [2] reported on 49 canine testicular tumors which he classified as follows: 32 seminomas, 15 Sertoli cell tumors, 2 malignant interstitial cell tumors. Metastases to the periaortic lymph nodes were associated with 1 interstitial cell tumor, with uncertain evidence of metastases in the second case; in 1 seminoma there was evidence of metastasis on microscopic examination of a lymph node. These are the only observations in the literature of metastases of canine testicular tumors. There was nodular hyperplasia

* This investigation was aided by a grant from the Committee on Research in Endocrinology, the National Research Council.
Received for publication, May 8, 1944.

of the interstitial cells in 12 cases which Innes did not regard as neoplastic.

The association of squamous metaplasia of the prostate with canine testicular tumors is of interest; this estrogenic stimulation is accompanied by other evidence of femaleness. Greulich and Burford [8] reported the findings in 3 dogs which had tumors located in cryptorchid testes with replacement of the cylindrical epithelium of the prostate with squamous cells; 2 of these dogs were sexually attractive to other males and had hypertrophy of the mammary papillae. The same syndrome has been reported by Zuckerman and co-workers.[9, 10] In an autopsy series of 243 dogs, 35 testicular tumors were observed: [9] seminomas, 17; adenocarcinomas, 15; interstitial cell tumors, 3. In 5 dogs there was estrogenic stimulation of the prostate, all associated with adenocarcinoma.

The experimental production of interstitial cell tumors in certain strains of mice by estrogenic administration was accomplished by Bonser and Robson [11] and by Hooker, Gardner and Pfeiffer.[12] These tumors are both benign, and malignant, metastasizing to regional lymph nodes, lung and above the kidney.[11,13,14] In this case estrogen produces atrophy of the involved testis to the Sertoli cell stage, with spermatogonia and occasionally spermatocytes being present.[11,12]

Methods

The high incidence of tumors in aged dogs has been repeatedly observed.[15–17] The tumors in the present study occurred without exception in very old animals; evidence of senility was the occurrence of lenticular opacities and wearing and discoloration of the teeth, especially of the incisors. The dogs were killed by electric current and the testes and prostates were weighed and then fixed in 10 per cent formalin. Frozen sections were made with staining for fat with sudan IV; paraffin sections were stained with hematoxylin and eosin. Chemical analyses were made on 23 of these tumors [18] and the results are correlated with the morphologic appearance in the present paper.

Results

No teratomas, sarcomas, or chorionic epitheliomas were found. All of the canine tumors were epithelial in nature and could be classified easily into four groups: interstitial cell tumors, seminomas, tubular adenomas and undifferentiated carcinomas. A statistical analysis is presented in Table I. All of the tumors were very vascular and it is known that such tumors bleed profusely when incised for biopsy.

Interstitial Cell Tumors

Most of the interstitial cell tumors were small, commonly 1 to 3 cm. in diameter, and consisted of a single nodule, yellow-orange, and of a consistency resembling brain. The weight varied from 1.87 to 24 gm. The nodules were discrete and were separated from the surrounding testis by a connective tissue capsule, several fibroblasts in thickness.

TABLE I

Statistics of Testicular Tumors of Dogs

Number of dogs with testicular tumors:			41
Number of testes involved:			58
Number of tumors:			64
Bilateral tumors: 17	Unilateral: 24		
Number of interstitial cell tumors:			33
Number of seminomas:			19
Number of tubular adenomas:			9
Number of undifferentiated carcinoma:			3
Multiple tumors in same testis:			6
Interstitial cell tumor and seminoma:			5
Interstitial cell and tubular adenoma:			1
State of germinal epithelium of the uninvolved testis:			
Interstitial cell tumor			
Normal: 11	Atrophy: 5		
Seminoma			
Normal: 5	Atrophy: 2		
Tubular adenoma			
Normal: 3	Atrophy: 2		
Undifferentiated carcinoma			
Normal: 3	Atrophy: 0		
State of the prostate:			
Interstitial cell tumor			
Normal: 3	Cystic hyperplasia: 18	Atrophy: 0	
Seminoma			
Normal: 2	Cystic hyperplasia: 7	Atrophy: 1	
Tubular adenoma			
Normal: 0	Cystic hyperplasia: 1	Atrophy: 4	
Undifferentiated carcinoma			
	Cystic hyperplasia: 2	Squamous metaplasia: 1	

On microscopy, it was seen that the cytoplasm was relatively much larger than the nucleus. The nucleus was basophilic and varied in size, sometimes small and dense, at other times larger and paler; it contained a single large nucleolus. The nucleus was always eccentric and usually polar. The cytoplasm was extensive, acidophilic and, containing many fat droplets, appeared foamy with vacuoles in a defatted section. Many of these tumors consisted of solid masses of cells, but in others, in addition, anastomosing cords often draped with macrophages

were prominent. These phagocytes laden with brown pigment were usually seen in this tumor. Areas of necrosis were common, with regions completely replaced by fat or hemorrhage or connective tissue. Viable areas of interstitial cells were always found and in no tumor was necrosis complete.

Seminomas

The seminomas were usually larger than interstitial cell tumors and their weight varied from 8 to 40 gm. In the gross they were white, firm and multinodular.

On microscopic examination a single type of tumor cell was found occurring either diffusely or confined to the seminal tubules; both the diffuse and tubular forms often occurred in the same testis. Among the 19 seminomas, 9 were diffuse, 5 tubular, 4 were both diffuse and tubular and 1 testis was nearly completely necrotic. The cell consisted of a single, large, basophilic nucleus with a small amount of weakly basophilic cytoplasm. The cells of canine and human seminomas resemble each other except that the canine tumor is more basophilic; the collections of lymphocytes which always accompany human seminomas are absent in the tumors of dogs.

Necrosis is less common in seminomas than in interstitial cell tumors. In the series of 19 seminomas, 16 were free from necrosis, 1 tumor was almost completely necrotic, another had extensive hemorrhage and a third had a nonlipoid type of vacuolated degeneration.

Tubular Adenomas

In the group of tubular adenomas the largest tumors occurred, weighing 4 to 408 gm. In the gross these tumors were white, solid, coarsely nodular and free from necrosis. They were bilateral in 4 dogs, in 1 of which the tumors were very large and situated in cryptorchid testes; a unilateral tumor was found in 1 dog.

Microscopically, tubular adenomas were found to consist of many closely packed tubules free from cyst formation. The tumors were not accompanied by Leydig cells and the stroma was sparse; they did not infiltrate but rather displaced the normal testicular tissue. The cells were long and irregular in shape and terminated in thread-like processes. In many instance the cells filled the tubules, extending from side to side and growing and clumping in the lumina. The cytoplasm was acidophilic and the nucleus was weakly basophilic.

Perhaps the most favorable situation in which to study normal Sertoli cells in the adult is in the testis of cryptorchidism. Here the tubules are not dilated and are separated by large groups of interstitial cells. The Sertoli cells are aligned along the basement membrane and

their cytoplasmic fibrils anastomose in the lumen. The nucleus of the Sertoli cells is strictly basal, arranged along the tubular basement membrane. In contrast to the normal Sertoli cells of undescended testes, the cells of tubular adenomas have lost their basal orientation in growing into the lumina of the tubules and there are no intratubular masses of Leydig cells; moreover, the nucleus is larger and less dense. Otherwise there is a close similarity of Sertoli cells of the cryptorchid to the principal cell of the tubular adenoma.

Undifferentiated Carcinoma

The group of undifferentiated carcinomas was small and consisted of 3 tumors, in which it was not readily possible to classify the carcinomatous cells with reference to the architecture of the normal testes. In 2 cases the tumors were large, weighing 24 and 47 gm., their cells resembling interstitial cells but with a low fat content. In both instances there was an associated senile cystic hyperplasia of the prostate. In a third case the tumor weighed 28 gm.; there was a diffuse pattern of large cells with a high fat content but the cells did not resemble interstitial cells nor were they arranged in tubules. This tumor was of interest since it was accompanied by estrogenic stimulation of the prostate, manifested by replacement of the cylindrical epithelium by multilayered squamous cells, many of which were lying free in the alveoli. The prostate weighed 11 gm., and projecting from one lateral surface there was a spheroidal bud 1.2 cm. in diameter.

The Fat Content of Testicular Tumors

The normal fat content of adult canine testes was found to be 1.47 ± 0.32 gm. per 100 gm. of wet weight.[18] On staining normal testes with sudan stain, fat was found in two types of cells, Leydig cells and Sertoli cells. Large amounts of fat were found in interstitial cell tumors (4 to 14.9 per cent) and in tubular adenomas (2.1 to 6.8 per cent) and this was reflected in the cells staining with sudan stain. The interstitial cell tumors were packed with fat droplets. There were free fat droplets in many of the lumina of the tubular adenomas as well as fat in the neoplastic cells. Also in the tubular adenomas, many of the tubules were surrounded by an areola of sudanophilic material in the peritubular connective tissue. Other tubules were free from fat in the lumina and cells, as well as in the surrounding areola. Seminomas did not stain with sudan dyes; their fat content varied from 0.2 to 0.6 gm. per cent. The undifferentiated tumor (fat content, 7.5 per cent), associated with estrogenic stimulation of the prostate, had masses of fat in the nuclei of the tumor cells.

Discussion

The determination of the neutral fat content of testicular tumors by chemical methods paralleled closely the staining with sudan dye. The sudan staining proved a useful aid in classification. The key is as follows:

1. Low fat content of cells—seminoma
2. High fat content with cells arranged in tubules—tubular adenoma
3. High fat content of vacuolated cells with absence of tubular arrangement—interstitial cell tumor

A single error was made in that an undifferentiated carcinoma of large cell type, producing estrogenic stimulation of the prostate, had a high fat content; there was absence of tubular arrangement and the cells did not resemble Leydig cells.

The general morphology of tubular adenomas, together with their high content of lipoids, prove that the neoplasms are tumors of Sertoli cells. In describing the sustentacular cells of the testis, Sertoli [19] discovered that these cells had a high fat content. Pick [20] first described a tumor of cylindrical epithelium, which he named "adenoma tubulare testiculare ovarii," in ectopic human testes and in the ovary of a pseudo-hermaphrodite. In the canine tubular adenomas the cells are larger, the alveoli smaller and more closely packed than in the Pick tumor in which the nuclei are basal. The cells do not grow into the lumina of the alveoli nor do they contain fat. Thus the similarity of these tumors is not close.

The large number of tumors with morphologic characteristics resembling cells of the normal testis with preservation of their normal lipoid relationships is evidence that the majority of the testicular tumors of dogs approach physiologic maturity—a point of significance from the standpoint of experimental and clinical cancer therapy. Moreover, none of the dogs with interstitial cell tumors had atrophy of the prostate, suggesting elaboration of androgens by the tumor; both tubular adenomas and seminomas were associated with prostatic atrophy (Table I) in several instances. The finding of a few large tumors with cells resembling interstitial cells but without fat is evidence that these tumors are physiologically more immature than the common type of interstitial cell tumors.

In the experimental production of interstitial cell neoplasm in mice by estrogen, moderate to severe atrophy occurred in the germinal epithelium of nontumorous regions of the testis. In two-thirds of the cases of spontaneous interstitial cell tumors the germinal epithelium was normal in appearance. In the undifferentiated carcinoma in this series which produced estrogen as manifested in squamous metaplasia

of the prostate, the germinal epithelium of the uninvolved regions of the testis was quite normal. This is evidence that the naturally occurring estrogens are different in kind or amount from those administered in the experimental production of interstitial cell tumors.

The close morphologic resemblance of canine and human seminomas suggests that these tumors have a comparable site of origin. The canine seminomas are much more frequently confined to tubules than the human tumors. The cell type in tubular and diffuse seminomas, however, is identical and these observations support the theory of Chevassu [7] that this tumor arises from germinal epithelium.

Summary

In a morphologic study of 64 tumors contained in 58 testes of 41 dogs, it was easily possible to classify the neoplasms in four groups: 33 interstitial cell tumors, 19 seminomas, 9 tubular adenomas, 3 undifferentiated tumors. Two of the undifferentiated tumors resembled interstitial cells but did not contain fat; a third tumor of this class had a high lipoid content but its cells did not resemble Leydig or Sertoli cells and was accompanied by squamous metaplasia of the prostate but with normal germinal epithelium in the testis. From a morphologic standpoint it may be inferred that most canine testicular tumors are physiologically mature; as further evidence, the prostates of all of the dogs with interstitial cell tumors were normal or enlarged while prostatic atrophy was observed in each of the other groups. Metastasis was not found in any case.

Staining of the tumors with sudan dyes proved useful in classification. Interstitial cell tumors had diffuse cells laden with fat. Many of the cells of tubular adenomas contained fat droplets; many of the alveoli were surrounded by a lipid areola. Seminomas contained no fat.

Evidence is presented that interstitial cell tumors are derived from Leydig cells, tubular adenomas from Sertoli cells and seminomas from germinal epithelium.

Moderate or severe atrophy of germinal epithelium has followed the experimental production of interstitial cell tumors with estrogen; two-thirds of the spontaneously developed interstitial cell tumors were accompanied by normal germinal epithelium.

REFERENCES

1. Schlotthauer, C. F., McDonald, J. R., and Bollman, J. L. Testicular tumors in dogs. *J. Urol.*, 1938, **40**, 539–550.
2. Innes, J. R. M. Neoplastic diseases of the testis in animals. *J. Path. & Bact.*, 1942, **54**, 485–498.

3. Kunze, A. Über Zwischenzellentumoren im Hoden des Hundes. *Virchows Arch. f. path. Anat.*, 1922, **240**, 144–165.
4. Pallaske, G. Beitrag zur Frage der "Zwischenzellentumoren" bei Tieren. *Virchows Arch. f. path. Anat.*, 1931, **281**, 856–870.
5. Peyron, A., Blanchard, and Salomon, L. Les tumeurs des cellules interstitielles du testicule. *Bull. Assoc. franç p. l'étude du cancer*, 1936, **25**, 427–450.
6. McDonald, J. R., Schlotthauer, C. F., and Bollman, J. L. The pathogenesis of seminoma of the testis. *Surgery*, 1938, **3**, 904–911.
7. Chevassu, M. Tumeurs du Testicule. G. Steinheil, Paris, 1906.
8. Greulich, W. W., and Burford, T. H. Testicular tumors associated with mammary, prostatic, and other changes in cryptorchid dogs. *Am. J. Cancer*, 1936, **28**, 496–511.
9. Zuckerman, S., and McKeown, T. The canine prostate in relation to normal and abnormal testicular changes. *J. Path. & Bact.*, 1938, **46**, 7–19.
10. Zuckerman, S., and Groome, J. R. The aetiology of benign enlargement of the prostate in the dog. *J. Path. & Bact.*, 1937, **44**, 113–124.
11. Bonser, G. M., and Robson, J. M. The effects of prolonged oestrogen administration upon male mice of various strains: development of testicular tumors in the Strong A strain. *J. Path. & Bact.*, 1940, **51**, 9–22.
12. Hooker, C. W., Gardner, W. U., and Pfeiffer, C. A. Testicular tumors in mice receiving estrogens. *J. A. M. A.*, 1940, **115**, 443–445.
13. Shimkin, M. B., Grady, H. G., and Andervont, H. B. Induction of testicular tumors and other effects of stilbestrol-cholesterol pellets in strain C mice. *J. Nat. Cancer Inst.*, 1941–42, **2**, 65–80.
14. Bonser, G. M. Malignant tumors of the interstitial cells of the testis in Strong A mice treated with triphenylethylene. *J. Path. & Bact.*, 1942, **54**, 149–154.
15. Goodpasture, E. W., and Wislocki, G. B. Old age in relation to cell-overgrowth and cancer. *J. M. Research*, 1915–16, **33**, 455–473.
16. Smith, L. W. Senile changes of the testis and prostate in dogs. *J. M. Research*, 1919, **40**, 31–51.
17. Huggins, C., and Clark, P. J. Quantitative studies of prostatic secretion. The effect of castration and of estrogen injection on the normal and on the hyperplastic prostate glands of dogs. *J. Exper. Med.*, 1940, **72**, 747–762.
18. Huggins, C., and Eichelberger, L. Studies on tumors of the testis. I. Water and electrolyte content of testicular tumors and of normal, cryptorchid, and estrogenized testis. *Cancer Research*, 1944, **4**, 447–452.
19. Sertoli, E. Sulla struttura dei canalicoli seminiferi dei testicoli. *Arch. per le sc. med.*, 1878, **2**, 107–146; 267–295.
20. Pick, L. Ueber Neubildungen am Genitale bei Zwittern. *Arch. Gynäk.*, 1905, **76**, 191–281.

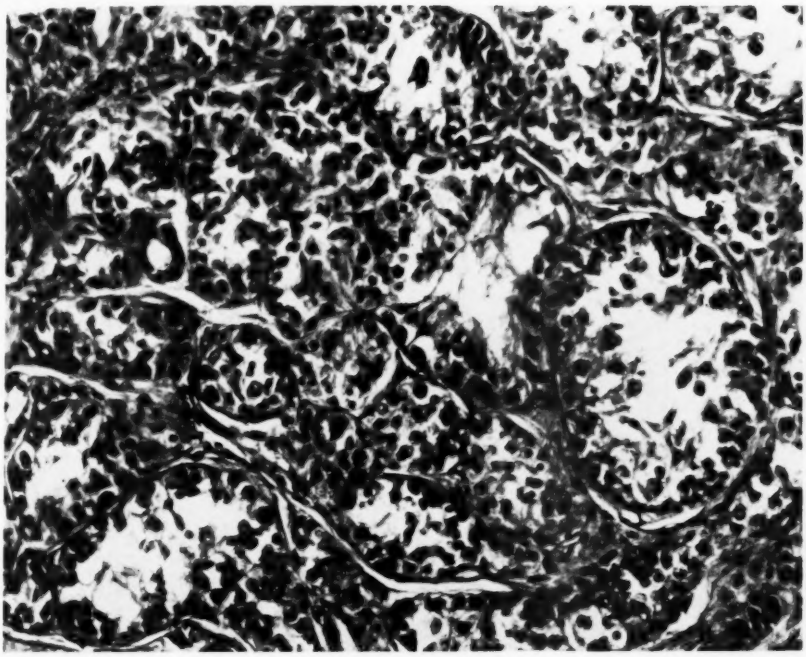
DESCRIPTION OF PLATES

PLATE 54

FIG. 1. Tubular adenoma of canine testis. × 250.

FIG. 2 Diffuse carcinoma of canine testis. × 475.

1



2

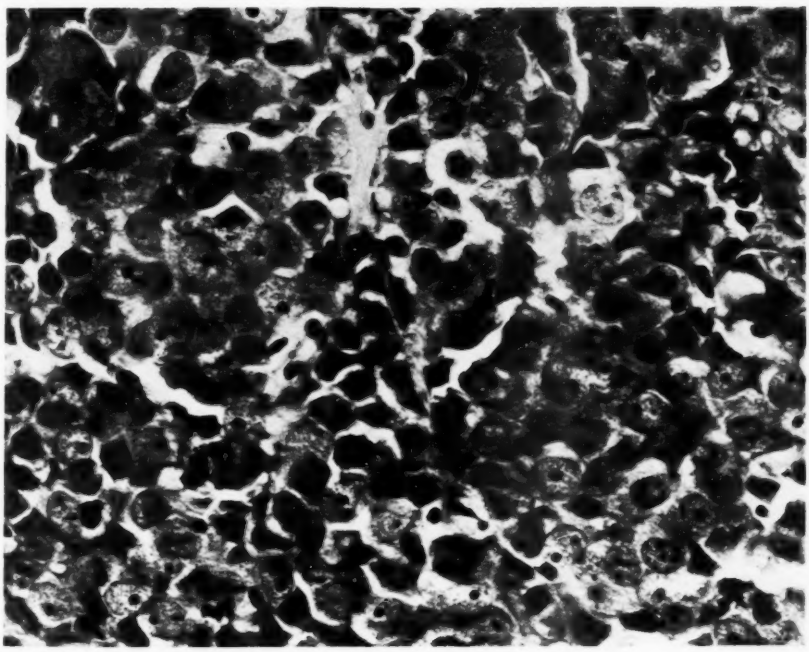


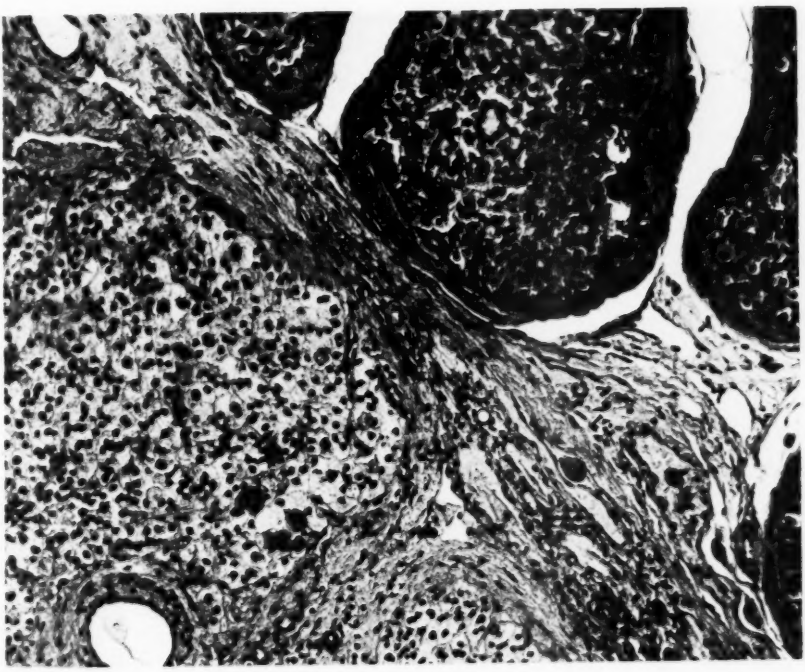
PLATE 55

FIG. 3. Interstitial cell tumor of canine testis; on the right is normal testicular tissue. × 520.

FIG. 4. Interstitial cell tumor and tubular seminoma of canine testis. × 160.

3

4



UNIV. OF MICH.

THE EFFECTS OF INHALED HEAT ON THE AIR PASSAGES AND LUNGS

An Experimental Investigation *

Alan R. Moritz, M.D., Frederick C. Henriques, Jr., Ph.D., and Regina McLean, B.Sc.
(From the Department of Legal Medicine, Harvard Medical School, Boston, Mass.)

Victims of conflagrations frequently sustain pulmonary injuries that are of equal or greater importance to survival than are the burns received on the surface of the body. In some instances the changes are confined to the upper air passages with little or no damage to the lungs. In some the larynx and trachea show little or no evidence of injury and profound pulmonary damage is sustained. In still others the entire tract is affected.

The precise cause of such respiratory injuries is usually obscure. In some conflagrations, as for example the Cleveland Clinic disaster in 1929, none of the victims was exposed to excessive heat and most of the deaths were believed to be due to the breathing of chemical irritants contained in the smoke that was given off by the burning of nitrocellulose x-ray films.[1] In many conflagrations, however, the victims have been exposed to heat as well as to smoke, and if respiratory injuries are sustained the question of whether they were due to thermal or to chemical trauma is likely to be raised.[2]

It is obvious that no two nonexperimental fires are likely to be exactly alike so far as their propensity for the elaboration of chemically irritating combustion products is concerned. A fire represents a series of chemical reactions, each of which may contribute a different group of potentially injurious gaseous, liquid, or solid irritants to the atmosphere. The nature and amount of these potentially injurious substances in smoke are determined in part by the composition of the material being burned, in part by the amount of oxygen that is available at different times during the conflagration, and in part by the temperatures that are attained. Among the more dangerous irritants likely to be encountered in smoke are aldehydes and acid anhydrides.

However numerous and variable the chemically injurious properties of smoke may be, it is apparent that the problem as a whole would be less formidable if a better understanding were had of the pathological characteristics of uncomplicated thermal injury of the lungs and air passages and of the temperatures that are necessary for their production. The opportunity to study such injuries in man in circumstances in which the possibility of concomitant chemical injury can be excluded is rare. It was decided, therefore, to investigate the problem under controlled conditions in the laboratory.

* Received for publication, November 20, 1944.

Experimental Methods

Eighteen dogs were used to investigate the effects of hot air on the respiratory tract and two pigs were used to acquire information regarding the relative vulnerability of the skin and the lining of the respiratory tract to certain types of exposure. All experiments were conducted under anesthesia induced by the intravenous or intraperitoneal injection of sodium pentathol.

In order to study thermal injuries of the lungs independently of any complications that might be caused by concomitant burning of the skin, the hot air was conveyed directly to the trachea by means of an insulated trans-oral cannula. The internal end of the cannula extended below the vocal folds of the larynx and the external end protruded from the mouth. In such experiments neither the skin nor the mucous membranes of the mouth or throat were exposed to heat.

Three types of inhalation experiments were performed. In the first the animals breathed ordinary hot air at different external temperatures. In the second the flame and combustion products from a blast burner were inhaled. In the third the animals breathed a mixture of live steam and air.

The external temperature of the air available for respiration in each type of experiment did not fluctuate to any considerable degree from breath to breath and was measured by a thermometer in some experiments and by a platinum-rhodium thermocouple in others. As the hot air was breathed the temperature within the internal air passages rose rapidly during inspiration and returned to, or almost to, normal during expiration. To measure these changes two thermocouples constructed of fine (36 or 40 gauge) wire were installed in the airway, one being placed where the inhaled air passed through the laryngeal end of a trans-oral cannula and the other at or near the bifurcation of the trachea. The former thermocouple was an iron-constantan junction and the latter was copper-constantan. The leads from both thermocouples were connected by means of a two-way switch with a Mohl galvanometer having a period of 0.2 seconds. The excursions of the galvanometer were observed directly and recorded manually. By changing the switch after each expiration the inspiratory temperature peaks in the upper and lower portions of the trachea were observed during alternate breaths.

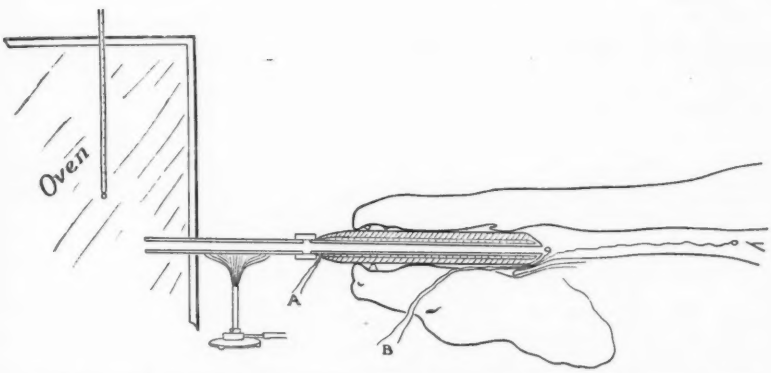
The upper thermocouple was fixed in the center of the laryngeal orifice of the cannula. The lower thermocouple, which was enclosed in a perforated cage to prevent it from coming in contact with tracheal mucous membrane, was inserted to the approximate level of the bifur-

cation by means of a long, slender wire. A certain amount of difficulty was encountered with the deep tracheal thermocouple because of the collection of mucus in the interstices of its protecting cage. It was necessary in some instances to interrupt experiments in order to clean this thermocouple.

Inhalation of Oven-Heated Air

In six experiments the animals breathed room atmosphere that had been heated in an oven (Text-Figs. 1 and 2).

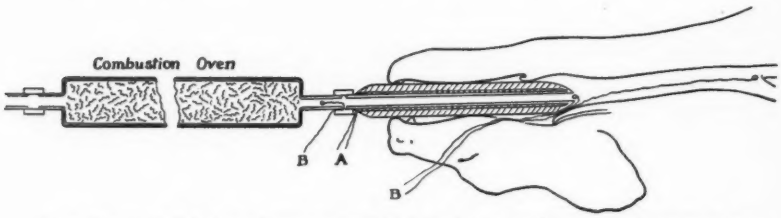
In three experiments (Text-Fig. 1) the temperature of the air as it left the oven was approximately 350° C. and the rate and amplitude of



Text-Fig. 1. Room atmosphere heated to 350° C. Hot air drawn from oven through an insulated trans-oral cannula by the spontaneous inspiratory efforts of the animal. (A) Leads to electrical heating unit in wall of cannula. (B) Leads to thermocouples in laryngeal end of cannula and lower portion of trachea.

respiration depended upon the spontaneous efforts of the animal. A short extension of the trans-oral cannula was inserted through the door of an electrically heated oven which had a capacity of approximately 0.8 cubic meters. A micro burner placed beneath the short metal extension tube tended to prevent cooling of the heated air as it was drawn through it and into the trans-oral cannula. The lining of the cannula was a glass tube having an internal diameter of 6 mm. By passing electricity through a coil of chromel wire which had been wrapped around the inner wall of the cannula, loss of heat during the passage of the air stream through the mouth was reduced. The outer wall of the cannula consisted of a layer of diatomaceous earth covered by lacquered cotton fabric. The internal end of the cannula was well insulated and was shaped to fit the laryngeal orifice snugly enough to prevent any leak of air around it.

In three experiments (Text-Fig. 2) respiration was passive rather than active and the hot air was pumped from a combustion oven into the trans-oral cannula at a rate of 16 strokes per minute, each stroke having a volume of 300 cc. The oven consisted of a bronze cylinder measuring 3 cm. in diameter and 45 cm. in length. To increase its

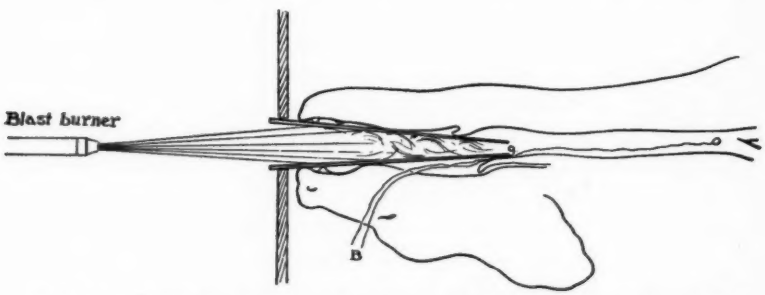


Text-Fig. 2. Inhalation of room atmosphere heated to 500° C. Hot air pumped from combustion oven through insulated trans-oral cannula into trachea. (A) Leads to electrical heating unit in wall of cannula. (B) Leads to thermocouples in outlet of oven, in laryngeal end of cannula and in lower portion of trachea.

heating efficiency the oven was packed with chrome steel shavings. The temperature of the air as it passed out of the oven and into the trans-oral cannula was approximately 500° C.

Inhalation of Flame

In five experiments (Text-Fig. 3) the only atmosphere available to the animal for respiration was the flame and combustion products of a glass blower's blast burner. In these experiments a funnel-shaped trans-oral cannula was used having an internal diameter of 2 cm. at its outer end and of 6 mm. at its laryngeal orifice. The external end of the cannula extended through a hole in a heavy sheet of asbestos board which was placed there to shield the animal's head from the flame.

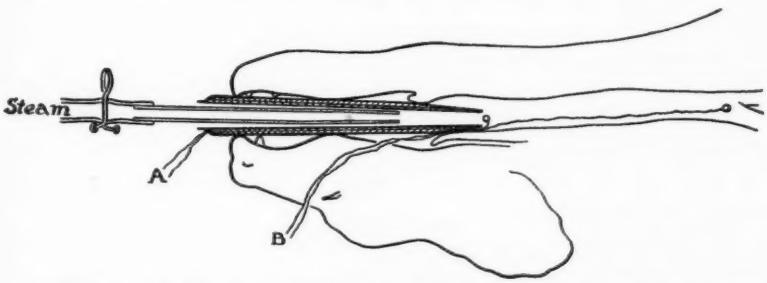


Text-Fig. 3. Inhalation of flame and combustion products from blast burner. Flame entered external end of cannula only during inspiration. (B) Leads to thermocouples in laryngeal end of cannula and in lower portion of trachea.

Approximately 20 cm. in front of the asbestos shield a blast burner was mounted in such a way that its flame could be projected directly into the external orifice of the cannula. The air-fuel mixture was adjusted so that the gas continued to burn throughout the length of the cannula. A shutter consisting of a disk of asbestos was mounted between the burner and the mouth of the cannula so that flame could be excluded from the cannula between inspirations. For the duration of each inspiration the shutter was opened and the flame allowed to enter the cannula.

Inhalation of Steam

In six experiments (Text-Fig. 4) the animals breathed steam which was generated in an 8 liter flask. Two exhaust tubes were passed through the stopper of the flask. The end of the first of these was introduced into the lumen of the trans-oral cannula and was kept closed



Text-Fig. 4. Inhalation of live steam. Steam entered cannula only during inspiration. (A) Leads to electrical heating unit in wall of cannula. (B) Leads to thermocouples in laryngeal end of cannula and in lower portion of trachea.

by means of a spring clamp except during the inspiratory phase of respiration. The wall of the tube was heated by electricity to prevent the condensation of moisture within it. The fit between it and the inside of the cannula was sufficiently loose so that air could be expelled through this compartment during expiration without having to pass back through the tube from which the steam was discharged. The free end of the second exhaust tube from the steam generator was submerged in a column of water through which the steam could bubble when the first tube was clamped. By regulating the height of the column of water in this trap it was possible to develop approximately the same amount of steam pressure within the flask during each interinspiratory interval. The pressure was so regulated that the release for 1 second of the spring clamp on the tube that opened into the cannula led to the discharge of approximately 250 cc. of live steam in some experiments and 400 cc. in others.

Results of Experiments

The number, kinds, and results of experiments performed to determine the effects of heat on the air passages and lungs are summarized in Table I.

Rate of Cooling of Inhaled Air

When superheated air was inhaled, the temperature recorded by both the laryngeal and the deep tracheal thermocouples rose throughout inspiration and fell during expiration. In each situation the highest point in the temperature curve was reached at or near the end of inspiration. As indicated in Table I, relatively dry gases lost most of their heat before reaching the lungs whereas the rate of heat loss from a steam-air mixture during its passage down the trachea was much less rapid. Another difference incident to the inhalation of dry and moist air was the contour of the temperature curves. When hot, dry air was inhaled, the temperature rose to a sharp peak and fell rapidly during expiration. When steam was inhaled, the curve described a plateau rather than a peak. It is likely that this character of the temperature curve during the inhalation of steam was due, in part at least, to the condensation of hot water on the thermocouples. This would tend to dampen their sensitivity and cause them to retain their heat longer than if they had remained dry.

In experiments 1 to 4 the air was inhaled directly from an oven in which the internal temperature was maintained at 350° C. Despite the fact that the airway between oven and larynx was short and that heat loss through its wall was reduced by auxiliary heating devices, the temperature of the air stream fell from 350° C. in the oven to between 159° and 182° C. by the time it reached the larynx.

In experiments 5 to 7 the air was pumped through the trans-oral cannula and into the trachea from a red-hot combustion oven. As the air left this oven its temperature was in excess of 500° C. and yet it fell to between 267° and 327° C. by the time it reached the laryngeal end of the cannula and to 50° C. by the time it reached the bifurcation of the trachea. Since the pump was regulated to discharge 300 cc. with each stroke and since the entire output of each stroke was forced into the trachea, the failure to develop high intrapulmonary temperatures could not be attributed to shallow breathing.

In experiments 8 to 12 the animals breathed the flame and combustion products of a glass blower's blast burner. As already indicated, the burner was so regulated that tongues of blue flame actually reached the laryngeal end of the cannula where air temperatures of between 327° and 550° C. were recorded during each inspiration. Despite this

TABLE I
Summary of Experimental Data

TABLE I

Summary of Experimental Data

Kind of atmosphere breathed	Animal numbers		Original pre-inspiratory temp. of air (approximate)	No. of breaths	Max. temperature recorded		Recovery period (hrs.)	Site and severity of injury		
					Laryngeal cannula	Lower trachea		Upper trachea	Lower trachea	Lungs
Air from drying oven (See Text-Fig. 1)	1	423	350° C.	46	182° C.	—	19	Mild	None	None
	2	420	350° C.	52	180° C.	—	19	Mild	None	None
	3	391	350° C.	103	159° C.	—	30	Mild	None	None
	4	390	350° C.	106	175° C.	—	Not killed	(Complete clinical recovery—no autopsy)		
Air from combustion oven (See Text-Fig. 2)	5	392	500° C.	60	267° C.	—	4	Mild	None	None
	6	432	500° C.	44	327° C.	50° C.	7	Moderate	None	None
	7	426	500° C.	22	291° C.	—	24	Mild	None	None
Flame from blast burner (See Text-Fig. 3)	8	431	———	17	———	135° C.	7	Moderate	Mild	None
	9	433	———	10	327° C.	51° C.	8	Severe	Moderate	Mild
	10	454	———	16	540° C.	100° C.	11	Severe	Mild	None
	11	455	———	24	550° C.	65° C.	24	Moderate	Mild	None
	12	405	———	14	510° C.	64° C.	Not killed	(Complete clinical recovery—no autopsy)		
Steam from generator (See Text-Fig. 4)	13	456	Over 100° C.	27	106° C.	59° C.	6	Moderate	Mild	None
	14	519	Over 100° C.	18	98° C.	79° C.	7	Severe	Moderate	None
	15	481	Over 100° C.	20	94° C.	53° C.	10	Severe	Severe	Severe
	16	475	Over 100° C.	15	99° C.	94° C.	10	Severe	Severe	Severe
	17	524	Over 100° C.	10	———	90° C.	24	Severe	Severe	Moderate
	18	522	Over 100° C.	12	———	75° C.	48	Severe	Severe	Mild

extraordinary attempt to produce a high intrapulmonic temperature, the highest thermocouple reading at the tracheal bifurcation was 135° C. (experiment 10). In the other four experiments the deep tracheal inspiratory peaks ranged between 51° and 100° C. The reason that the deep tracheal temperature peaks in experiment 10 were so much higher than those obtained in the other experiments with the inhalation of flame was not discovered. Actually no. 10 was the first experiment that was performed in the flame series and so far as is known the same experimental method was followed throughout.

Although the respiration was spontaneous in these experiments, the inhalation of combustion products caused rapid, deep breathing which should have predisposed to the attainment of maximal intrapulmonary temperature levels.

In experiments 13 to 18 a mixture of live steam and air was inhaled. The inspiratory peaks recorded at the laryngeal opening of the cannula ranged between 94° and 106° C. and the temperatures recorded by the deep tracheal thermocouple ranged between 53° and 94° C. Although the temperature recordings in both situations were probably altered by condensation of moisture on the thermocouples, it was obvious that steam had more heat to give up and that it gave it up more slowly than did hot, dry air.

Effects on Animals

As already indicated, it was the purpose of these experiments to expose the air passages and lungs to heat without causing concomitant injury of the skin so that any pathological changes that occurred could be attributed to the inhaled heat independently of any secondary effects that might result from thermal injury of the surface of the body.

Skin. Actually the mildest thermal exposures used in the inhalation experiments were more than sufficient to cause severe injury to the skin. Every animal included in Table I would have sustained extensive cutaneous injury if the skin had been exposed for more than a few minutes to an atmosphere as hot as that which entered the trans-oral cannula. It was observed in pilot experiments on dogs and pigs that air (room atmosphere) temperatures as low as 300° C. produced severe injury of unprotected skin after a 30 seconds' exposure. Mixtures of steam and air at 100° C. destroy epidermis within a few seconds. The destructive effect on a dog's epidemis of a 1 second exposure to steam is shown in Figure 4.

Pharynx and Larynx. Early in the investigation it was found that if animals were to survive an injurious thermal exposure long enough to permit the development of reactive changes in the lower air passages,

it was necessary for the primary impact of the hot air to be below rather than above the larynx. If the air is inhaled in a normal fashion and at a high enough temperature to damage the lower air passages, it is likely to cause death from obstructive edema of the laryngopharynx and larynx (Fig. 5) before sufficient time has elapsed for the development of reactive changes in the deeper portions of the respiratory tract.

In a series of exploratory experiments not included in Table I, the internal end of the trans-oral cannula was modified in such a manner that the first impact of the hot atmosphere during inspiration occurred in the pharynx rather than below the vocal folds of the larynx. In these pilot experiments it was found that several breaths of dry air delivered into the pharynx at a temperature of 300° C. or of steam delivered at 100° C. caused such a severe local edema within a few hours that the animals died of obstructive asphyxia.

The mucosa of the pharynx and larynx of the dog is not uniformly susceptible to the development of edema. The tongue and the laryngeal surface of the epiglottis can sustain severe injury without the occurrence of appreciable swelling. However, a relatively short exposure of the laryngopharynx, the pharyngeal surface of the epiglottis, or of the laryngeal ventricles results in the development of a massive gelatinous mucosal edema which tends to obstruct the airway within a few hours. Severe burning of the lower portion of the larynx or of the trachea may be survived with remarkably little reduction in the patency of the airway.

Thermal Injuries of the Respiratory Tract (Sublaryngeal). It may be seen in Table I that pulmonary injury occurred in none of the 7 animals that breathed oven-heated air, in only 1 of the 5 animals that inhaled flame from a blast burner, and in 4 of the 6 animals that inhaled live steam. In the remaining animals primary injury caused by the inhalation of superheated atmosphere was confined to the upper air passages.

Thermal Tracheitis. Animals 1 to 4 breathed air directly from the 350° C. drying oven and developed a mild catarrhal reaction of the upper respiratory tract. Although the reaction was minimal so far as microscopical appearance was concerned, the copious outpouring of mucus during the first 12 to 18 hours of the recovery period provided evidence of its occurrence. The surface epithelium of the tracheal mucosa remained intact and there was neither edema nor cellular exudation.

Animals (5 to 7), into whose tracheas air had been pumped from a 500° C. combustion oven, developed an acute tracheitis for a distance of 8 to 12 cm. below the larynx. In addition to edema of the subepi-

thelial stroma and evidence of increased secretory activity of mucous glands, animal 6 disclosed a circumscribed patch of mucosal ulceration where the stream of hot air from the orifice of the improperly aligned cannula first struck the posterior wall of the trachea. In none of the animals of this group was there recognizable injury of the lower portion of the trachea, the bronchi, or the lungs.

Animals 8 to 18 breathed flame or live steam and developed tracheitis ranging in intensity from moderate to severe. Although the most severe injuries invariably occurred in the upper portion of the trachea of these animals, catarrhal and in some instances membranous inflammation was prolonged beyond the bifurcation.

Mucosal necrosis with desquamation of surface epithelium occurred in all instances where the blast of hot atmosphere first struck the tracheal wall. In the case of injuries designated as moderately severe the mucosal ulceration was localized. Tracheitis designated as severe was characterized by extensive and, as a rule, completely circumferential ulceration.. In both types of injury the necrotic mucosa desquamated and the denuded surface acquired a thick covering membrane of necrotic epithelium, fibrin, and exudative cells. In the case of severe injuries large casts of necrotic mucosa were expelled by coughing during the first 12 hours of the recovery period.

In injuries designated as moderate the edema was confined to that part of the tracheal wall that lay within the cartilaginous rings. In severe injuries peritracheal edema of varying intensity was encountered. In some instances it was confined to the neck and in others it extended into the mediastinum and even into the peribronchial and perivascular connective tissue of the central portions of the lungs.

Pulmonary Injury. One of the 5 animals that breathed flame and 4 of the 6 animals that breathed a mixture of live steam and air disclosed evidence of primary thermal injury of the lungs in addition to severe injury of the upper respiratory passages.

The portions of the lungs most vulnerable to thermal injury were those centrally located alveolar ducts and communicating alveoli that had the shortest and most direct connections with the large bronchi at the hilum. Atmosphere not hot enough to damage the mucosa of the large bronchi or the alveoli of the peripheral portions of the lungs may still be hot enough to cause edema and hemorrhage of centrally located alveoli. In 3 of the 5 animals in which thermal pneumonitis was recognized, the primary pulmonary injuries were confined to the central portions of the lobes.

In the mildest form of primary thermal injury of the lungs the changes were confined to the terminal portions of centrally located

respiratory bronchioles, their alveolar ducts and the adjacent air sacs. In the early stage of such a lesion the walls of the affected alveoli and alveolar ducts were thickened, in part by edema and in part by hyperemia, and there was an escape of deeply acidophilic serous fluid into the lumen. In more severe injuries of this type, extravasated erythrocytes and fibrin were present in the escaped fluid. Where sufficient heat had reached the lungs to produce such changes, it was usual to find edema of the connective tissue around the larger blood vessels and air passages even though the mucosa of the latter showed little or no evidence of damage. Although there was evidence of capillary injury, as indicated by extravasated erythrocytes, no thrombosis was observed.

An example of what might be characterized as relatively mild central pneumonitis due to inhalation of hot atmosphere is shown in Figure 3. It may be seen that the large bronchi are contracted and show no evidence of mucosal injury. The peripheral pulmonary parenchyma is normal. The central parenchyma is relatively airless due in part to hyperemia and atelectasis and in part to intra-alveolar edema. Occasional small bronchi are obstructed by masses of aspirated mucosal débris. This débris came from the upper portion of the trachea which was extensively ulcerated. A higher magnification of the consolidated central portion of the lung is shown in Figures 11 and 12.

In two animals (15 and 16) which inhaled steam, the pulmonary injury was characterized as severe. In these there was extensive and generalized destruction of the bronchial mucosa. Instead of the usual post-mortem contraction (Fig. 3) the ulcerated bronchi remained widely patent (Fig. 10). Although there was generalized pulmonary edema and hyperemia in these animals, the massive interstitial hemorrhage and interstitial swelling in the hilar regions led to a particularly striking form of central consolidation. Such injuries were observed only in animals that had inhaled steam and the impression was gained that the water-laden atmosphere had sufficient residual heat to cause capillary injury even after it had reached the most outlying portions of the lungs. It was noteworthy that these two animals survived for 10 hours despite the severity of their pulmonary injuries.

Focal patches of atelectasis and emphysema were encountered in animals that had sustained primary pulmonary injury and in those that had not. In some instances these disturbances were obviously due to the aspiration of mucus or mucosal débris from the upper air passages. In others the cause of the obstruction was not discovered. In no instance was it felt that obstruction of the small air passages of this type interfered significantly with pulmonary ventilation.

Bronchopneumonia was observed as early as 8 hours after inhala-

tion of superheated atmosphere and occurred in animals that had, as well as in those that had not, sustained recognizable thermal injury of the lungs. When recognizable thermal injury was confined to the upper air passages it was inferred that pulmonary infection was secondary to the aspiration of mucosal débris.

Bronchopneumonia was observed only in animals that had sustained severe tracheal injury. When the thermal injury was limited to the upper air passages the pneumonia tended to be peripheral rather than central. When primary thermal pulmonary injury had been sustained the pneumonic patches were central and peripheral. Bacteriological examination of the intra-alveolar exudate disclosed a mixed flora with cocci in clumps and chains predominating.

There was no reason to infer that any of the animals whose thermal lesions were designated as mild or moderately severe and limited to the upper air passages would have suffered any significant or prolonged respiratory disturbance. No animal in the entire series of experiments died during the inhalation of hot air. One animal (no 5) died 4 hours after exposure of obstructive laryngeal edema due to defective insulation of the laryngeal end of the trans-oral cannula. None of the animals that inhaled flame died, although it is likely that 9 and 10 would have died of infection if they had not been killed. In both instances there was extensive ulceration of the tracheal mucosa. Animal 12 inhaled 14 breaths of flame and was allowed to survive. This animal was used several months later in another experiment at which time there was neither clinical nor pathological evidence of residual pulmonary injury. It was thought that none of the dogs that breathed steam would have survived, with the possible exception of animal 13. Two of these animals died with massive pulmonary edema within the first 12 hours. Two were obviously suffering from respiratory distress when they were sacrificed after recovery periods of 24 and 48 hours respectively.

Although no animals known to have sustained a thermal pneumonitis were allowed to survive longer than 48 hours after exposure, the pathological changes observed in the lungs within 48 hours permitted certain inferences as to the ultimate fate of these animals if a longer survival period had been allowed. With severe thermal injury of the lungs, the edema incident to widespread injury of the pulmonary capillary bed might be expected to cause death from asphyxia or circulatory failure within 12 hours. Regardless of the mildness of a primary thermal injury of the lung, if the inhaled air was hot enough to cause direct pulmonary damage, the associated tracheal injury might be expected to predispose the animal to widespread infection of the respiratory tract.

That such an infection would be likely to lead to the formation of pulmonary abscesses was indicated by its observed severity in those portions of the lung that were obstructed by necrotic masses of aspirated mucosal débris.

Discussion

As indicated in the introductory paragraphs, this investigation was undertaken to determine the pathological characteristics of pulmonary lesions caused by the inhalation of hot atmosphere. Although the relative immunity of the lungs to thermal injury might have been anticipated if due consideration had been given to the thermodynamics of the situation, these factors were not given adequate consideration until after the experiments had been performed.

Why is it that air hot enough to burn the skin of the face can be inhaled without causing damage to the trachea or lungs? Surely the mucosa of the air passages is more delicate and should therefore be more vulnerable to thermal injury than the epidermis. The explanation of the unanticipated results of this investigation lies in the fact that the quantity of heat that can be stored in the volume of gas that constitutes a breath is remarkably small. At any given air temperature the number of calories that can be transferred to the respiratory tract incident to the inhalation of a breath of hot air is limited by the volume of that breath, whereas in the case of the surface of the body where convection currents are capable of bringing a practically unlimited volume of hot air in contact with the skin, an infinitely greater caloric transfer can occur for each unit of surface area exposed.

An illuminating illustration of this difference is provided by calculating the heat transfer that could occur in the respiratory tract if air were inhaled at 142° C. Let it be assumed that the amount inhaled with each breath would be sufficient to increase the pulmonary volume by 500 cc., that the air was dry when inhaled and saturated with moisture when exhaled and that it was cooled to body temperature by the time it was exhaled. The cooling of one such breath from 142° to 38° C. would release approximately 13 calories of heat energy in the body. Theoretically this amount of heat would be sufficient to raise the temperature of 1 gm. of tissue by approximately 13° C. Actually the liberation of this amount of heat would produce no net change in the temperature of the respiratory tract because it would be offset by a loss of 13 calories due to the evaporation of the 23 mg. of water that would be required to saturate that amount of dry air. This is not to imply that the inhalation of air heated to 142° C. would be necessarily harmless. Desiccation or even burning might occur near the portal of entry even though no net change in the temperature of the respiratory tract occurred.

Although there are probably no nonexperimental circumstances in which thermal injury of the respiratory tract can be sustained without the occurrence of concomitant and more severe burning of the exposed surface of the body, there is great variation in the potentiality of different kinds of atmosphere for the production of thermal injury. This variation does not depend so much on differences in heat capacity of the inhaled gases (or material suspended in them)as it does on differences in their capability for heat output through condensation of moisture as the gases are cooled within the body.

Thus, to use the same type of calculation for moist air as was used for dry air, let it be assumed that a mixture of equal parts of steam and air was inhaled at a temperature of 125° C. Such an atmosphere will cause severe burning of the skin within a second. If an amount of such a mixture sufficient to increase the lung volume by 500 cc. were inhaled and if it were cooled to 38° C. before being exhaled, approximately 300 mg. of water would be condensed in the respiratory tract. The heat energy liberated, incident to the condensation of this amount of water, would be approximately 175 calories. In addition to this, approximately 12 calories would be liberated incident to the cooling of the gases and 26 calories incident to the cooling of the condensed water, bringing the total transfer of heat to the respiratory tract in excess of 200 calories. This probably represents a greater transfer of heat than would occur incident to the inhalation of dry air at conflagration temperatures.

Another matter for consideration is the extent to which the results of these experiments can be applied to man. Is it fair to assume that man and dog would be similarly affected by the inhalation of superheated atmosphere under nonexperimental conditions? If hot air were inhaled under ordinary conditions, it seems likely that the relatively long trachea of the dog would make him less vulnerable to thermal pulmonary injury than man. The air would have to travel farther in the dog and would have a better opportunity to give up its heat before reaching the lungs. It seems highly probable, however, that the elaborate precautions that were taken in the animal experiments to prevent loss of heat from the inhaled air as it was conducted to the larynx would more than compensate for the shortness of the human trachea.

Conclusions

1. In experiments on dogs it was observed that only when the original temperature of the air was high enough to produce almost instantaneous burning of the skin and upper respiratory mucosa was

there sufficient residual heat in the air reaching the lungs to cause pulmonary injury.

2. Within the range covered by this investigation no type of thermal pulmonary exposure was encountered which was immediately incompatible with life. A thermal exposure sufficient to injure the lungs was more than enough to cause a rapidly fatal obstructive edema of the glottis. Only when the larynx was protected against heat did animals survive long enough to develop the characteristic lesions of thermal pneumonitis.

3. At any given temperature moist air has more heat to give up than does an equal volume of dry air and is accordingly more likely to cause thermal injury of the respiratory tract.

4. Inhalation of dry or moist hot air may destroy the upper tracheal mucosa without causing primary thermal injury of the lungs. In the dog a severe thermal tracheitis predisposes to the development of bronchopneumonia.

5. The most vulnerable portion of the lung to thermal injury is the central parenchyma where the respiratory bronchioles and alveoli have the shortest and most direct connection with the primary bronchi.

6. In instances of mild thermal injury of the lungs the centrally located alveoli were the seat of hemorrhagic edema even though there had been insufficient heat to cause recognizable injury of the bronchial mucosa or of the more peripherally located air sacs.

7. In instances of severe thermal injury of the lungs there was extensive destruction of bronchial mucosa, dilatation of large and small bronchi, hemorrhagic edema of the peribronchial connective tissue and generalized hyperemia and hemorrhagic edema of the peripheral as well as of the central pulmonary parenchyma.

REFERENCES

1. The Disaster at the Cleveland Hospital Clinic, Cleveland, Ohio, on May 15, 1929. Proceedings of a Board of the Chemical Warfare Service, U. S. Government Printing Office, Washington, 1929.
2. Mallory, T. B., and Brickley, W. J. Management of the Cocoanut Grove burns at the Massachusetts General Hospital. Pathology: with special reference to the pulmonary lesions. *Ann. Surg.*, 1943, 117, 865–884.

[*Illustrations follow*]

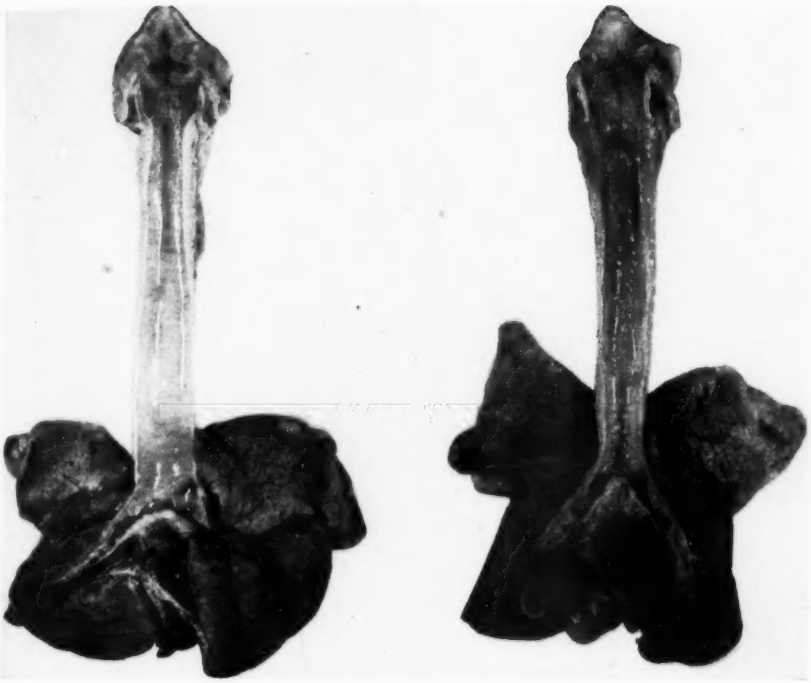
DESCRIPTION OF PLATES

PLATE 56

FIG. 1. Thermal laryngitis and tracheitis without pulmonary injury. Photograph of respiratory tract of dog 24 hours after inhalation of flame. Sufficient heat had been conducted through the wall of the cannula to cause a mild degree of laryngeal edema which may be recognized by the bilateral olive-shaped mucosal protrusions from the ventricular recesses. There was extensive destruction of the mucosa of the upper trachea, diminishing rapidly to a mild catarrhal inflammation in the lower third. No abnormality of the bronchi or lungs of this animal was recognized.

FIG. 2. Thermal tracheitis and pneumonitis. Photograph of respiratory tract of dog 10 hours after inhalation of steam, showing severe tracheobronchitis with dilatation of the bronchi. There is a central hemorrhagic pneumonitis with generalized pulmonary edema and hyperemia.

FIG. 3. Primary thermal pneumonitis. Photomicrograph of lower lobe of dog's lung 24 hours after inhalation of steam. Although there was severe tracheitis, the primary and secondary bronchi showed remarkably little change. Evidence of pulmonary injury was confined largely to the central portions of lobes and consisted of hyperemia, edema and partial atelectasis. × 9.



1 2

3

UNIV. OF MICH.

PLATE 57

FIG. 4. Steam burn of dog's skin. Photomicrograph showing complete necrosis and beginning separation of epidermis from corium 24 hours after a 1 second exposure to live steam. × 80.

FIG. 5. Obstructive edema of glottis due to inhalation of hot air. Photograph of pharynx and glottis of a dog 4 hours after inhalation of hot (500°C.) air. In this experiment the first impact of the hot air was above rather than below the glottis.

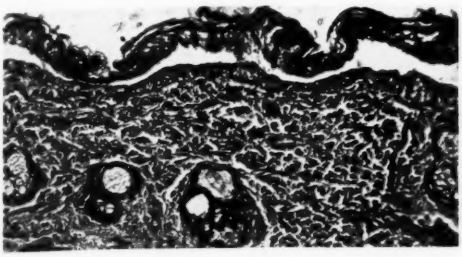
FIG. 6. Thermal tracheitis. Photomicrograph of the mucosa of upper portion of trachea 24 hours after inhalation of flame from blast burner. The necrotic superficial epithelium has desquamated and the underlying stroma is desiccated. Beneath the zone of desiccation there is severe edema. The appearance of the lower tracheal mucosa of this animal is shown in Figure 8. × 55.

FIG. 7. Cast of tracheal mucosa expelled approximately 8 hours after inhalation of steam. × 160.

FIG. 8. Catarrhal tracheitis. Photomicrograph showing stromal edema and distended goblet cells in mucosa of lower trachea 24 hours after inhalation of flame. The appearance of the mucosa of the upper trachea of this animal is shown in Figure 6. × 305.

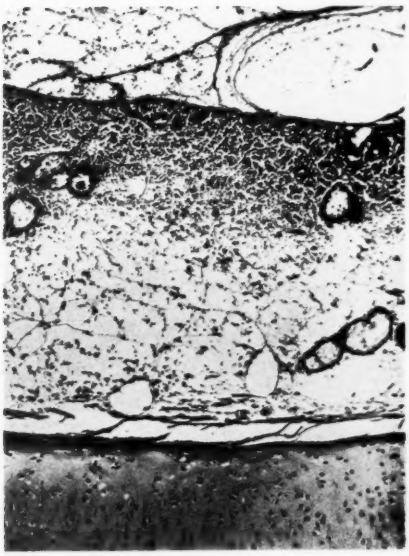
FIG. 9. Normal lung 24 hours after inhalation of flame. Photomicrograph showing wall of large bronchus and adjacent alveoli. Figures 6 and 8 show condition of trachea of the same animal.

4

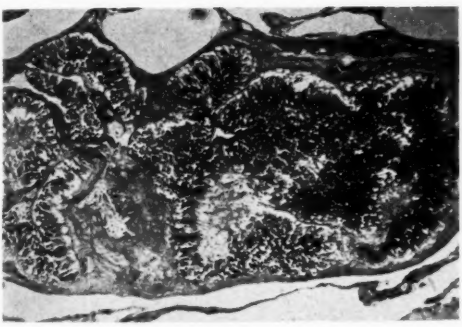


5

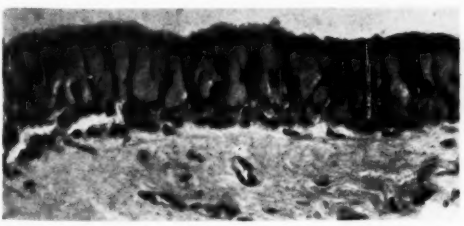
6



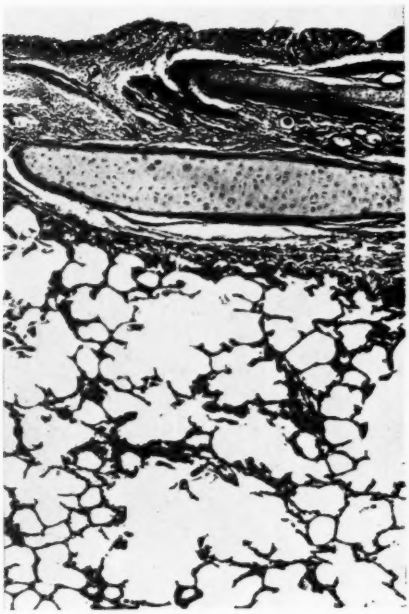
7



8



9



Moritz, Henriques and Mc Lean

Effects of Inhaled Heat

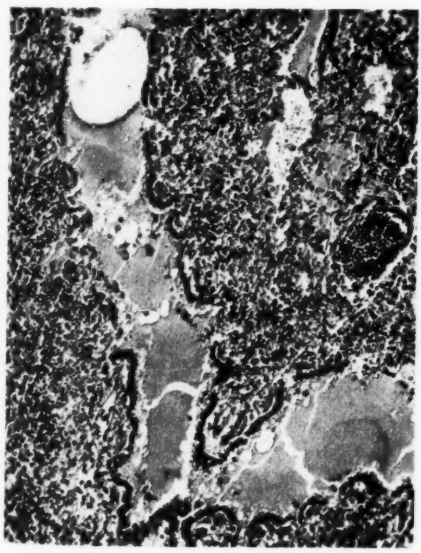
PLATE 58

FIG. 10. Severe thermal pneumonitis. Photomicrograph of section near hilum of lung showing hemorrhagic edema of central interstitial tissue, dilatation of ulcerated bronchi, and generalized edema and hyperemia of alveoli 10 hours after inhalation of steam. The macroscopical appearance of the lungs of this animal was similar to that shown in Figure 2. × 7.

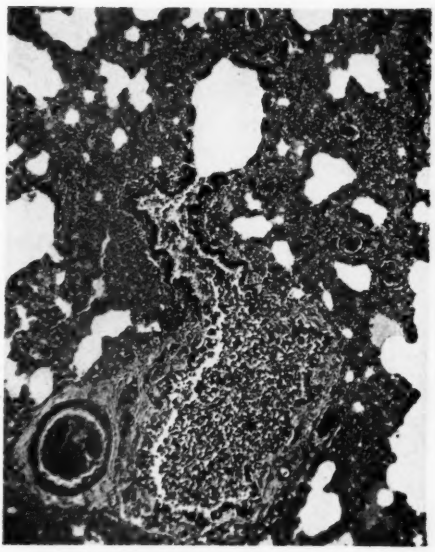
FIG. 11. Thermal pneumonitis. Photomicrograph showing edema of terminal bronchiole in central portion of lung 24 hours after inhalation of steam. The partially collapsed adjacent alveoli are hyperemic and contain many extravasated erythrocytes. × 120.

FIG. 12. Thermal pneumonitis. Photomicrograph showing terminal bronchiole partially obstructed by aspirated mucosal débris and exudate 24 hours after inhalation of steam. There is partial atelectasis of the surrounding alveoli, associated with hyperemia and edema. × 65.

10



11



12

Moritz, Henriques and Mc Lean

Effects of Inhaled Heat

A MORPHOLOGICAL STUDY FOLLOWING THE INTRAVENOUS ADMINISTRATION OF GELATIN SOLUTIONS TO DOGS *

ROBERT P. MOREHEAD, M.D., and J. M. LITTLE, Ph.D.

(From the Departments of Pathology, Physiology and Pharmacology of the Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.)

A group of studies has been carried out recently in an effort to determine the suitability of gelatin solutions as nonhematogenous blood substitutes. Ivy, Greengard, Stein, Grodins and Dutton,[1] and also Lawson and Rehm [2] have studied the effectiveness of gelatin solutions in the treatment of hemorrhagic shock in dogs. The use of such solutions in preventing shock induced by trauma to leg muscles and by tourniquets was reported by Kleinberg and his collaborators.[3] The permeability of damaged capillaries to gelatin has been studied by Little and Wells.[4] The effect of intravenous gelatin upon plasma retention, the rate of urinary excretion and the total circulating red cell volume in normal dogs and in dogs with diminished plasma volumes were reported by Little and Dameron.[5,6] *In vivo* conglutination of erythrocytes following the intravenous administration of gelatin has been studied by Miller and Little.[7]

Before a blood substitute may be accepted as a therapeutic agent, it must be demonstrated conclusively that there are no permanent pathological changes following the repeated administration of the substance. Hueper [8] has reported the occurrence of degenerative vascular disease in dogs following the intravenous administration of gelatin solutions of high concentration. In his experiments, initial injections of 2 cc. of a 10 per cent solution of gelatin were given daily, and both the concentration and dose were gradually increased until finally 120 cc. of a 25 per cent solution were being administered. The lesions found in these animals were primarily of a sclerotic type and were noted in the aorta and in the coronary and renal arteries. They consisted principally of a marked fibrous thickening of the intima with medial necrosis and hyalinization. Hueper concluded that the lesions present in these animals were directly attributable to parenterally introduced solutions of gelatin, although he noted further that the lesions were not so striking as those found following the injection of other macromolecular substances.

In view of the fact that gelatin solutions of extremely high concentration were used in Hueper's [8] experiments, it was deemed advisable to make a detailed pathological study of a number of dogs following the repeated administration of gelatin solutions in concentrations more closely approaching those likely to be used clinically.

* Supported in part by a grant from the Knox Gelatine Co.
Received for publication, April 20, 1944.

Material and Methods

Eight mongrel dogs were given 12 daily intravenous injections, each injection being equivalent to 1 per cent of their body weight, of either 6 per cent or 4 per cent gelatin in aqueous 0.9 per cent sodium chloride solution (Table I). One dog (no. 5) was given only 2 injections of the 6 per cent gelatin solution. The gelatin was prepared by hydrolysis of beef bone and had an iso-electric point near pH 5. At varying intervals following the last injection, the animals were killed with chloroform or by the intravenous injection of sodium pentobarbital.

Autopsies were performed immediately and sections were removed

Table I

Summary of Daily Injections of Gelatin Solution with Each Administration Equivalent to 1 Per Cent of the Body Weight

Dog no.	Weight	Days receiving 6% gelatin	Days receiving 4% gelatin	Days intervening between last injection and sacrificing
1	17 kg.	12	—	2
2	15 kg.	12	—	19
3	10 kg.	12	—	10
4	15.5 kg.	12	—	24
5	6.5 kg.	2	—	1
6	14.5 kg.	8	4	3
7	18.0 kg.	8	4	7
8	21.5 kg.	8	4	17
9	22.0 kg.	8	4	25

from all organs, special care being taken to obtain adequate material from the vascular, hematopoietic and nervous systems. Three sections were taken from each of the anatomical divisions of the aorta, since this organ has been especially incriminated in previous experimental work. The material removed for microscopical study was fixed in Zenker's fluid-formaldehyde solution and in a 4 per cent solution of neutral formaldehyde. All tissues were cut at 6 μ and stained routinely with hematoxylin and eosin, Mallory's connective tissue stain, and Masson's trichrome stain, and by Weigert's method for elastic fibers.

Results

Except in dog 1, the pathological findings were confined to the aorta, the kidneys and the coronary arteries. For purposes of description, the lesions may conveniently be grouped according to the structures affected.

Aorta. The most common abnormal findings in the aorta were nodular thickenings in the intima of the abdominal portion of the vessel (dogs 2, 6, 7, 8 and 9). These nodules were composed of deli-

cate elastic lamellae and fibrous tissue. There was thickening of the intima in the immediate vicinity of the nodules and occasionally in situations quite remote from them.

Degenerative changes in the elastic tissue of the media were noted in all of the dogs, although the degree of degeneration varied greatly and in many instances was minimal. The areas left vacant by the elastic tissue were filled in with smooth muscle and connective tissue cells. This process was practically confined to the ascending aorta and arch of the vessel and increased in severity as the aortic valve was approached. Dogs 1, 4, 7 and 8 showed, in addition to these changes, degeneration of elastic tissue and collagen which resulted in cyst formation. Also, in dogs 5 and 7 a scar was seen in the ascending aorta and calcium deposits were present in the immediate vicinity of the valve.

Aortic bacterial endocarditis associated with *Dirofilaria immitis* infestation was seen in dog 1. The liver and spleen were enlarged and clear fluid was present in the serous sacs.

Coronary Arteries. In only one dog (no. 7) could a lesion be demonstrated in the coronary arteries. This abnormal finding consisted of a localized thickening of the subendothelial connective tissue of the intima in one of the larger coronary radicles, with some encroachment upon the lumen of the vessel.

Kidneys. Five of the dogs (nos. 3, 4, 5, 6 and 8) showed chronic interstitial nephritis. In three animals (nos. 2, 7 and 9) no pathological changes could be demonstrated in the kidneys. Dog 1 presented a typical focal nephritis, identical with that seen in the kidneys of persons suffering from bacterial endocarditis.

Discussion

When the data from these experiments are examined, it is readily seen that there is no correlation between the pathological lesions exhibited by these animals and the administration of gelatin. The lesions are in general those of vascular degeneration and repair. In most instances sufficient time did not elapse between the beginning of the experiment and its termination to permit the development of lesions of the type found. Furthermore, if the lesions were produced by gelatin there should be some correlation between the duration of the experiment and the degenerative and reparative processes.

Bacterial endocarditis associated with elastic tissue and collagenous degeneration of the aorta and cyst formation was present in dog 1, which was sacrificed 14 days after the beginning of the experiment. Elastic tissue destruction and replacement fibrosis were seen in dog 5, although this animal was killed 3 days following the initial injection

of gelatin. Dogs 2 and 3 presented neither cystic nor sclerotic lesions, although 31 and 22 days respectively had elapsed since the beginning of the experiment.

Intimal thickening was noted in five animals which were sacrificed within 15 days (dog 6) to 37 days (dog 9) after the initial gelatin injection.

Spontaneous arteriosclerosis in the dog has been described, and it even has been suggested that only animals below the age of 2 years should be employed in experimental work involving a consideration of the vascular system.[9]

Chronic nephritis is a common finding in dogs and Bloom [10] has recently demonstrated that in the great majority of instances it is interstitial in type. In 200 animals dying of various causes, he found that 108 showed focal interstitial nephritis.

Bacterial endocarditis is a rare disease in dogs. However, it has been seen in association with *Dirofilaria immitis* infestation,[11] although most animals which are infected with this parasite show no abnormalities of the heart valves. Faust [12] stated that he has never observed the condition.

Spontaneous medionecrosis with or without cyst formation and smooth muscle replacement has not been described in the dog, although Rottino [13] has demonstrated degenerative lesions with similar smooth muscle and connective tissue changes in 95 of 210 unselected human aortas. Further, it is significant that the medionecrosis in human aortas described by Erdheim [14] and Moritz,[15] and of frequent occurrence in our work, has not been described in the dog.

Finally, it is interesting to note that during the course of our investigation Koop and his associates [16] concluded that there were no permanent pathological changes occurring in normal dogs as a result of repeated infusions of gelatin over long periods of time.

Summary and Conclusions

Nine mongrel dogs were given daily injections of gelatin solutions in concentrations most likely to be used clinically. These animals were sacrificed after varying periods of time, and degenerative and reparative changes, confined for the most part to the aorta, were found in the vascular system. Inflammatory lesions present in the kidneys of more than half of the dogs were similar to the interstitial nephritis commonly seen in these animals and recently emphasized by Bloom.[10]

If the degenerative, reparative and inflammatory changes present in these animals had resulted from the parenteral administration of gela-

tin solutions, one would expect to find some correlation between these processes and the duration of the experiment. No such correlation could be noted.

It is apparent from recent work that many investigators have not taken into account spontaneous aortic disease in the dog in evaluating their experimental results. In an attempt to clarify the problem further, it was deemed advisable to sacrifice for comparison a series of supposedly healthy mongrel dogs and to carry out carefully executed anatomical studies. The lesions seen in the control animals did not differ fundamentally from those seen in the ones which had received injections of gelatin. Since spontaneous vascular disease in the dog does not appear to be generally recognized, it was thought that the vascular lesions in both groups of animals were of sufficient interest to warrant an additional and more detailed report.[17]

REFERENCES

1. Ivy, A. C., Greengard, H., Stein, I. F., Jr., Grodins, F. S., and Dutton, D. F. The effect of various blood substitutes in resuscitation after an otherwise fatal hemorrhage. *Surg., Gynec. & Obst.*, 1943, **76**, 85–90.
2. Lawson, H., and Rehm, W. S. The relative value of various fluids in replacement of blood lost by hemorrhage, with special reference to the value of gelatin solutions. *Am. J. Physiol.*, 1943, **140**, 431–438.
3. Kleinberg, W., Remington, J. W., Eversole, W. J., Overman, R. R., and Swingle, W. W. The effectiveness of plasma, gelatin and saline transfusions in preventing shock induced by leg muscle trauma and tourniquets. *Am. J. Physiol.*, 1943, **140**, 197–204.
4. Little, J. M., and Wells, H. S. Capillary permeability to intravenously administered gelatin. *Am. J. Physiol.*, 1943, **138**, 495–498.
5. Little, J. M., and Dameron, J. T. Plasma retention, urinary excretion and effect upon circulating total red cell volume of intravenous gelatin in normal dogs. *Am. J. Physiol.*, 1943, **139**, 438–445.
6. Little, J. M., and Dameron, J. T. Plasma retention, urinary excretion and effect upon circulating total red cell volume of intravenous gelatin in dogs with diminished plasma volume. *Am. J. Physiol.*, 1944, **140**, 636–638.
7. Miller, R. E., and Little, J. M. Studies on the *in vivo* conglutination of erythrocytes following the intravenous administration of gelatin solutions. *J. Cell. & Comp. Physiol.*, 1943, **22**, 127–130.
8. Hueper, W. C. Experimental studies in cardiovascular pathology. V. Effects of intravenous injections of solutions of gum arabic, egg albumin and gelatin upon the blood and organs of dogs and rabbits. *Am. J. Path.*, 1942, **18**, 895–933.
9. Strauch, C. Zur Kenntnis der spontanen Arterienveränderungen beim Hunde mit besonderer Berücksichtigung der Arteriosklerose. *Beitr. z. path. Anat. u. z. allg. Path.*, 1915–16, **61**, 532–549.
10. Bloom, F. Classification and pathology of renal disease in the dog. *Arch. Path.*, 1939, **28**, 236–245.
11. Brown, H. W. Personal communication.
12. Faust, E. C. Personal communication.

13. Rottino, A. Medial degeneration of the aorta. *Arch. Path.*, 1939, **28**, 377–385.
14. Erdheim, J. Medionecrosis aortae idiopathica cystica. *Virchows Arch. f. path. Anat.*, 1930, **276**, 187–229.
15. Moritz, A. R. Medionecrosis aortae idiopathica cystica. *Am. J. Path.*, 1932, **8**, 717–734.
16. Koop, C. E., Riegel, C., Vars, H. M., Ratcliffe, H. L., Parkins, W. M., and Lockwood, J. S. II. Observations on toxicity and elimination of gelatin. *Am. J. M. Sc.*, 1943, **205**, 876–877.
17. Morehead, R. P., and Little, J. M. Changes in the blood vessels of apparently healthy mongrel dogs. *Am. J. Path.*, 1945, **21**, 339–355.

CHANGES IN THE BLOOD VESSELS OF APPARENTLY HEALTHY MONGREL DOGS *

ROBERT P. MOREHEAD, M.D., and J. M. LITTLE, Ph.D.
(From the Departments of Pathology, Physiology and Pharmacology, the Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.)

As a result of anatomical studies made in a group of dogs which had received gelatin solutions parenterally, it was concluded that there were no permanent pathological lesions in these animals which could be directly attributed to the gelatin.[1] Degenerative and reparative processes in the vascular system were of frequent occurrence, but since there was no correlation between the duration of the experiment and the type of lesion found, it was concluded that the changes were spontaneous and had no relation to the experimental procedure employed. This view was strengthened further by the fact that the vascular changes in the experimental animals were identical morphologically with those which have been described in a large percentage of human aortas removed from persons dying of various causes.

A review of the literature reveals that many investigators have not appreciated fully the existence of spontaneous vascular disease in the dog. It is the purpose of this paper to point out certain pathological changes occurring in this animal which have recently received considerable attention in human material but have not been described in the dog.

MATERIAL AND METHODS

Eleven mongrel dogs were chosen at random from the City Dog Pound and were killed immediately by shooting or by the intravenous administration of sodium pentobarbital. Five puppies, approximately 6 months old, and 3 additional ones, 10 days old, were sacrificed by the same methods. It was thought permissible to include 9 additional dogs utilized in another experiment, since it was concluded from that study that there were no abnormal alterations in the tissues which could be attributed to the experimental procedure.[1]

Autopsies were performed immediately and numerous blocks were removed from all organs, special care being taken to include several blocks from each of the anatomical divisions of the aorta, the heart, and the kidneys. The tissue was fixed in Zenker's fluid-formaldehyde solution and also in a 4 per cent solution of neutral formaldehyde. All material was sectioned at 6 μ and stained routinely with hematoxylin and eosin, Mallory's connective tissue stain, and Masson's trichrome stain, and by Weigert's method for elastic fibers.

* Received for publication, April 20, 1944.

Results

The lesions found in these animals were confined for the most part to the vascular system and kidneys, and consisted of degenerative and reparative changes in the former and inflammatory lesions in the latter. For purposes of description the anatomical changes have been classified according to the organs involved.

Aortic Lesions

The aortic lesions were of four principal types:

1. *Focal loss of elastic tissue with grouping of smooth muscle cells* was most marked in the ascending aorta and arch of the vessel. It was displayed in varying degrees by all animals, but was much less prominent in the 10-day-old puppies. The lesions were characterized by thinning of the elastic lamellae and, in many instances, by interruption of their continuity. Some areas were practically devoid of elastic tissue (Fig. 1). The lamellae adjacent to these areas were compact and the interlamellar spaces were greatly narrowed. The areas devoid of elastic tissue were filled in by groups of spindle-shaped cells possessing oval or spherical nuclei which were surrounded by an abundant cytoplasm (Figs. 2 and 3). In some instances the nuclei were hyperchromatic, while in others the chromatin was granular and tended to accumulate at one pole of the cell. In tissues prepared by Masson's trichrome method, the cytoplasm of the spindle cells stained a deep red, and blue-staining collagenous fibers radiated in all directions among the cells. In some instances bundles of collagenous connective tissue fibers were seen, and occasionally they formed minute scars (Fig. 4). The spindle-shaped cells appeared for the most part to be smooth muscle cells, but a varying number of fibroblasts were scattered throughout the areas. Many of the cells were arranged in a circular manner, but not infrequently they pursued an oblique or longitudinal direction (Fig. 3).

2. *Medial necrosis with cyst formation* was pronounced in only 2 dogs, but was seen in varying degrees in 8 animals. It did not occur in either group of puppies. The lesions were most marked in the ascending aorta and arch of the vessel. In the focal areas of degeneration the tissues normally composing the media had disappeared and had been replaced by a basophilic substance which not infrequently contained cells in various stages of degeneration (Fig. 5). In certain instances a delicate fibrillar material could be demonstrated within the cysts, and occasionally intracystic collagenous deposits were noted.

3. *Intimal thickening with splitting and reduplication of the internal elastic lamina* was present in 14 dogs and was confined to the abdominal portion of the aorta. In 7 animals nodular formations projected into the lumen of the vessel (Fig. 6), while in the remainder only a localized

thickening was noted (Fig. 7). Smooth muscle and connective tissue cells and varying amounts of collagen were present in addition to the elastic tissue. Changes of this type could be demonstrated in all groups of animals, including the 10-day-old puppies (Fig. 8). Five dogs showed localized thickening of the intima with marked fibrosis and hyalinization. In 3 animals sections prepared from the aorta near its bifurcation showed thickening and disruption of the internal elastic lamina with fibrous thickening of the intima and nodule formation (Fig. 9).

In every animal certain sections revealed a separation of the lamellae of the intima resulting in the formation of irregular cystic spaces (Fig. 10). This phenomenon was best seen in the ascending aorta and arch of the vessel.

4. *Localized areas of hyalinized collagenous tissue with calcification* were present in 5 dogs and were confined to the ascending aorta (Figs. 11 and 12). They were seen in the intima and media of the vessel, and in no instance was the process extensive. This finding was not seen in any of the puppies.

Sclerosis of the Coronary Artery

In only one animal was there any evidence whatsoever of sclerosis of the coronary artery. In this instance the abnormality was limited to a minimal fibrous tissue thickening of the intima of one of the larger coronary radicles.

Bacterial Endocarditis

One dog presented lesions identical with those ordinarily seen in cases of bacterial endocarditis in man. The heart and liver were enlarged and weighed 250 and 960 gm. respectively. Free fluid was present in all of the serous cavities and there was an increase in density in the more dependent portions of the lungs.

When the heart was opened, large numbers of *Dirofilaria immitis* were found in the right side of the organ. Numerous friable vegetations were present on the aortic valve (Fig. 13), and considerable hyalinized fibrous tissue had been deposited within it. The microscopical picture was identical with that seen in the human disease, and Gram-positive cocci were present in large numbers within the vegetations. The liver and lungs of this animal were congested and there were areas of focal nephritis (Fig. 14).

Chronic Interstitial Nephritis

Inflammatory kidney disease was present in 11 dogs and was characterized by plasmacytic and lymphocytic infiltration. The lesions were confined for the most part to the interstitial tissue, and large groups of

inflammatory cells surrounded the tubules, blood vessels and glomeruli (Figs. 15 and 16). A generalized increase in fibrous tissue was noted in most instances, and in some instances small areas of renal parenchyma were destroyed and replaced by fibrous tissue. The arterioles in these areas were frequently sclerotic. Occasionally small amounts of calcium salts had been deposited in the degenerated areas (Fig. 17). Minimal hyaline deposits in the intima of the larger renal vessels were quite common and occurred in kidneys which were otherwise normal with the same frequency as in those which were the seat of interstitial nephritis. These changes were confined to adult dogs.

Discussion

A review of the literature reveals that canine pathology has, in general, been a greatly neglected subject. In recent years, however, several attempts have been made to study arteriosclerosis in the dog. Most workers in this field restricted the use of the term arteriosclerosis to those instances in which there were localized areas of degeneration in the intima associated with fatty deposits and plaque formation. Other authors employed the term in the broad sense "as a collective and descriptive term covering the changes without active inflammation, that affect the smoothness, thickness, uniformity, homogeneity of tissue, elasticity of the vessel wall and the maintenance of a normal lumen." [2]

Köllisch [3] examined the aortas from 100 dogs and, employing the term to include all degenerative vascular changes, stated that he found arteriosclerosis in 23 of them.

Strauch [4] studied the aortas from a group of dogs ranging from 1 to 5 years of age and also from a group of older dogs. Restricting his use of the term to those instances in which intimal degeneration, with fatty deposits and plaque formation, was demonstrable, he was able to demonstrate true arteriosclerosis in only one animal in the younger group. In the older group he found arteriosclerosis to be a fairly common lesion. This author also found in young and in old dogs a widespread degeneration of the outer layers of the media, associated with scar formation and some thickening of the intima. These changes were confined to the abdominal portion of the vessel. "Stripes" or "ridges" were present in many of the vessels, and Strauch attributed them to the state of contraction of the media. These changes were present in the aorta of nearly every animal examined, and were most marked near the origin of the vessel.

In a recent study of human aortas removed routinely at autopsy, Rottino [5] found medial degeneration in approximately one-half of them.

The majority of the vessels examined showed only simple muscle loss, but elastic tissue degeneration and cyst formation were present in some instances. These changes have not been described previously in the dog, but a large number of our animals presented lesions morphologically identical with those seen in the human material studied by Rottino. The most common lesions consisted of focal loss of elastic tissue and grouping of smooth muscle cells, with or without associated collagenous deposits. In many instances the smooth muscle cells appeared to be actually increased in number, although it was difficult to ascertain this with certainty. Medial necrosis with cyst formation, identical with that described for the human aorta,[6-8] was seen. Hyalinization of the intima and media, associated with calcium deposits, was found in 5 of our animals. It has been pointed out by Nieberle [9] that this sometimes occurs in the first part of the aorta of dogs.

Localized areas of intimal thickening characterized by splitting and reduplication of the internal elastic lamella were present in a large number of animals and were seen even in puppies only 10 days of age. It has been noted previously [2] that this is a common finding in lower animals. Folding of the intima with separation of the elastic lamella was seen in every animal and was most marked in the thoracic portion of the vessel. This, however, is probably due to post-mortem contraction of the media.

It can readily be seen that spontaneous aortic disease is very common and occurs in animals of all ages. However, medial necrosis associated with cyst formation and collagenous deposit is seen only in adult dogs. Pronounced lesions in vessels other than the aorta are extremely rare, and in only one instance was intimal thickening seen in the smaller vessels. Hyaline deposits were frequent in the intima of the larger vessels in the kidney, but there was no thickening of the intima or encroachment upon the lumen of the vessel.

Chronic interstitial nephritis was common in these animals, and the changes were morphologically identical with those recently described by Bloom.[10] Bloom has pointed out that other types of nephritis, such as pyelonephritis and glomerulonephritis, are extremely rare in the dog.

One of our animals showed bacterial endocarditis which was morphologically identical in every way with that seen in man. The accidental finding of bacterial endocarditis in dogs is extremely rare. One cannot say with certainty what part infestation of the blood stream with *Dirofilaria immitis* played in the development of this disease. A careful search of the literature revealed no reference to bacterial endocarditis associated with *Dirofilaria immitis* infestation in dogs. Faust [11] stated

that he had never observed the two conditions in the same animal. However, in a personal communication Brown [12] stated that he had observed animals similar to the one described here.

Summary

In 28 mongrel dogs sacrificed for purposes of anatomical study, there was found to be a high incidence of degenerative changes in the aorta and of inflammatory lesions in the kidney. The aortic lesions consisted of focal loss of elastic tissue with grouping of smooth muscle cells, medial necrosis with cyst formation, intimal thickening with splitting and reduplication of the internal elastic lamina, and localized areas of hyalinized collagenous connective tissue with calcification. Atheromatous plaques were not present in this material, although localized intimal thickenings were of common occurrence and were seen in all groups of animals, including very young puppies. Hyaline deposits were frequently present in the intima of the larger branches of the renal artery, and in one instance intimal thickening was noted in a coronary vessel.

Chronic interstitial nephritis was present in more than one-half of the fully grown animals, and one dog suffered from bacterial endocarditis associated with *Dirofilaria immitis* infestation.

Unless the investigator familiarizes himself with the vascular changes which commonly occur in the dog, he is apt to attribute such changes erroneously to the experimental procedure employed.

References

1. Morehead, R. P., and Little, J. M. A morphological study following the intravenous administration of gelatin solutions to dogs. *Am. J. Path.*, 1945, **21**, 333–338.
2. Fox, H. In: Cowdry, E. V. Arteriosclerosis in Lower Mammals and Birds; Its Relation to the Disease in Man. The Macmillan Co., New York, 1933, p. 154.
3. Köllisch, P. Zur pathologischen Anatomie und Äetiologie der sogenannten Atherosklerose der Arterien bei den Haustieren. Inaugural Dissertation, Bern, J. L. Stich, Nürnberg, 1910.
4. Strauch, C. Zur Kenntnis der spontanen Arterienveränderungen beim Hunde mit besonderer Berücksichtigung der Arteriosklerose. *Beitr. z. path. Anat. u. z. allg. Path.*, 1915–16, **61**, 532–549.
5. Rottino, A. Medial degeneration of the aorta. *Arch. Path.*, 1939, **28**, 377–385.
6. Erdheim, J. Medionecrosis aortae idiopathica cystica. *Virchows Arch. f. path. Anat.*, 1930, **276**, 187–229.
7. Moritz, A. R. Medionecrosis aortae idiopathica cystica. *Am. J. Path.*, 1932, **8**, 717–734.
8. Klotz, O., and Simpson, W. Spontaneous rupture of the aorta. *Am. J. M. Sc.*, 1932, **184**, 455–473.

9. Nieberle, K. Über Atherosklerose beim Papagei. *Verhandl. d. deutsch. path. Gesellsch.*, 1930, **25**, 291–295.
10. Bloom, F. Classification and pathology of renal disease in the dog. *Arch. Path.*, 1939, **28**, 236–245.
11. Faust, E. C. Personal communication.
12. Brown, H. W. Personal communication.

[*Illustrations follow*]

DESCRIPTION OF PLATES

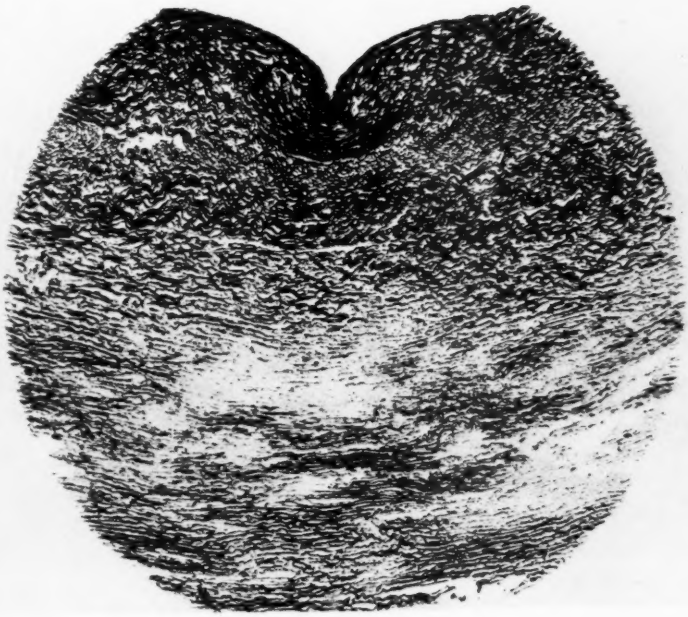
PLATE 59

FIG. 1. Focal loss of elastic tissue. Weigert's elastic tissue stain. × 110.

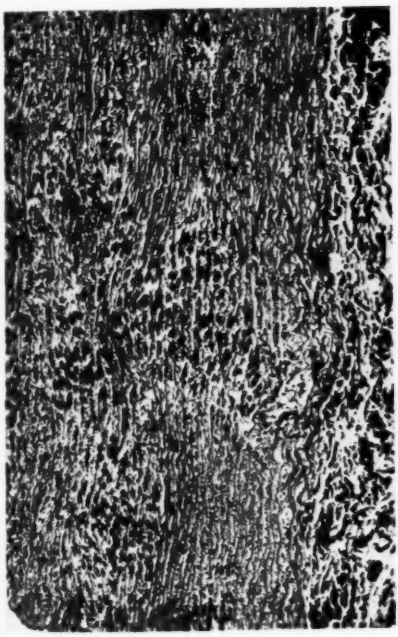
FIG. 2. Focal loss of elastic tissue with grouping of smooth muscle cells. Masson's trichrome stain. × 110.

FIG. 3. An area of grouped muscle cells from the same aorta as that used for Figure 2. × 250.

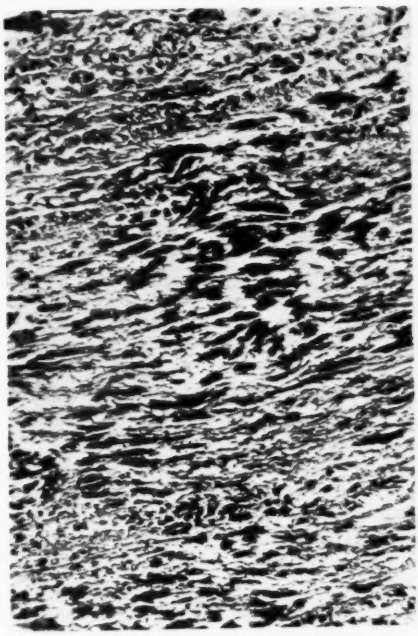
1



2



3



Morehead and Little

Blood Vessels of Apparently Healthy Dogs

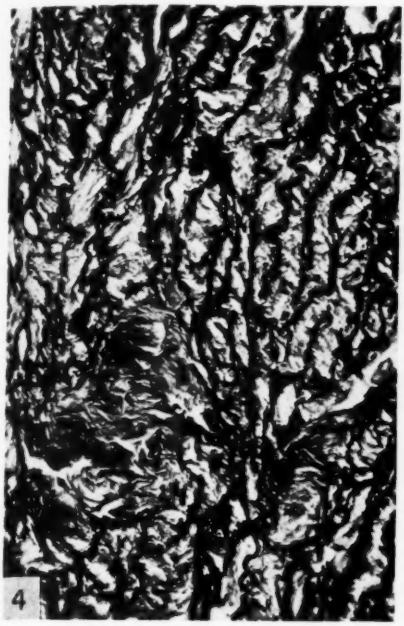
PLATE 60

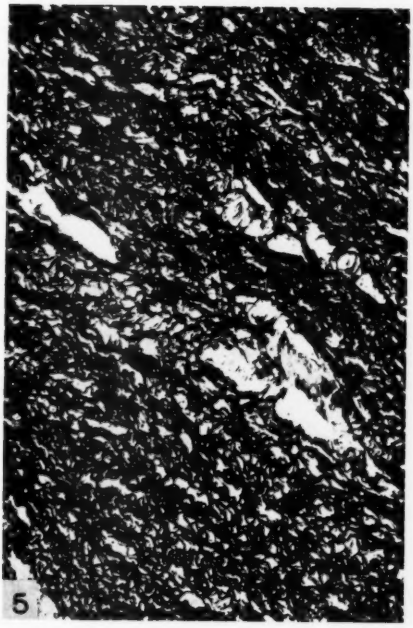
FIG. 4. Collagenous deposits in the media. Masson's trichrome stain. × 250.

FIG. 5. Medial necrosis with cyst formation. × 110.

FIG. 6. Splitting and reduplication of the internal elastic lamina with nodule formation. Weigert's elastic tissue stain. × 60.

FIG. 7. Thickening of the intima with splitting and reduplication of the internal elastic lamina but without nodule formation. Weigert's elastic tissue stain. × 60.





Morehead and Little

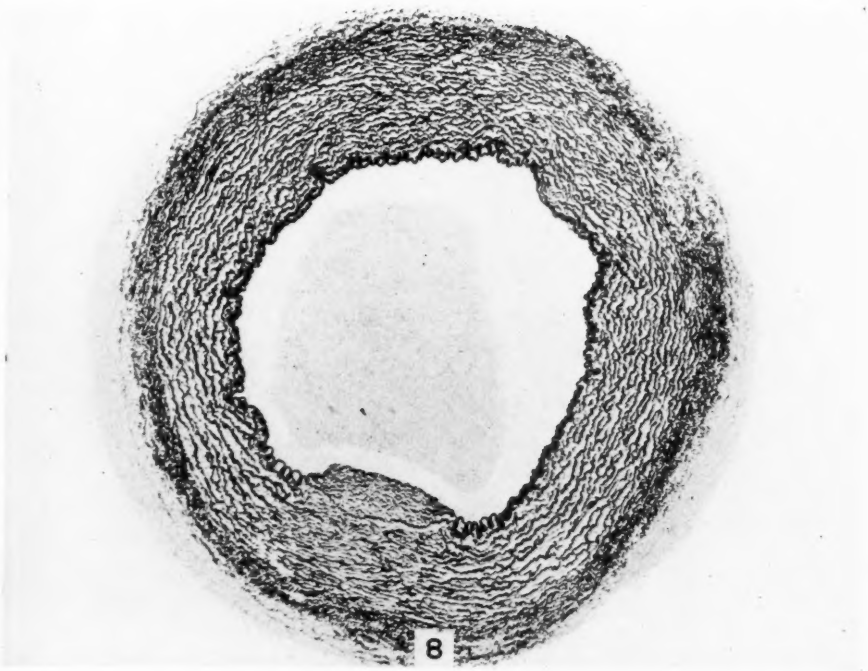
Blood Vessels of Apparently Healthy Dogs

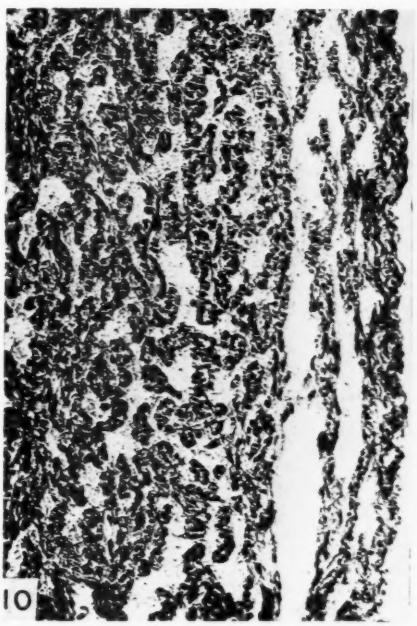
PLATE 61

FIG. 8. The same process as shown in Figure 6, occurring in a 10-day-old puppy. Weigert's elastic tissue stain. × 60.

FIG. 9. Thickening and disruption of the internal elastic lamina with fibrous thickening of the intima and nodule formation. This section was taken from the aorta near its bifurcation. Weigert's elastic tissue stain. × 250.

FIG. 10. Separation of the lamellae of the intima resulting in the formation of irregular cystic spaces. Masson's trichrome stain. × 110.





Morehead and Little

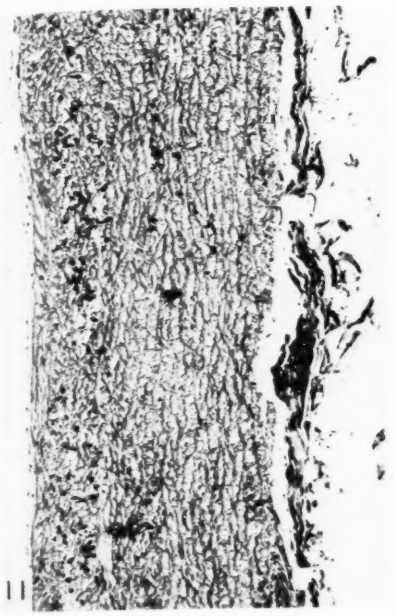
Blood Vessels of Apparently Healthy Dogs

PLATE 62

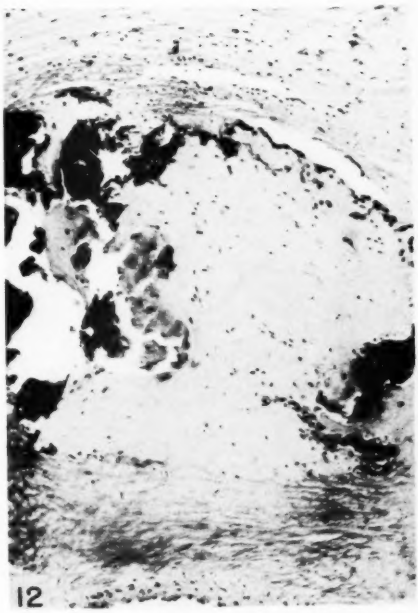
FIG. 11. Calcium deposits in the intima and media of the aorta in the vicinity of the aortic valve. Masson's trichrome stain. × 60.

FIG. 12. Fibrous thickening of the intima and media of the aorta with calcification. This section was from the ascending aorta. Hematoxylin and eosin stain. × 110.

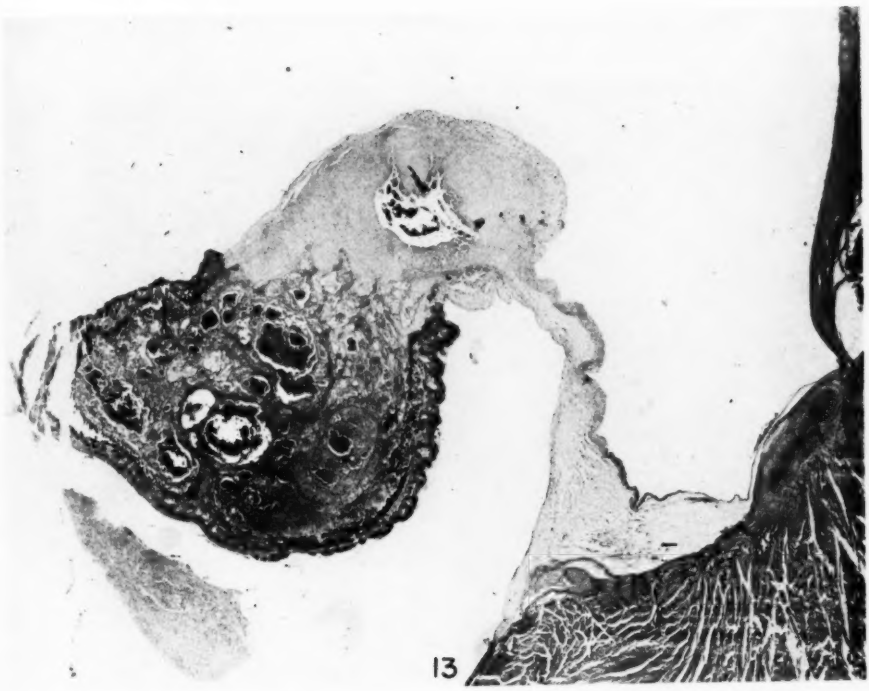
FIG. 13. Low power photomicrograph of the aortic valve showing a large vegetation. Cartilage can be seen at the base of the valve. Hematoxylin and eosin stain. × 11.



11



12



13

Morehead and Little

Blood Vessels of Apparently Healthy Dogs

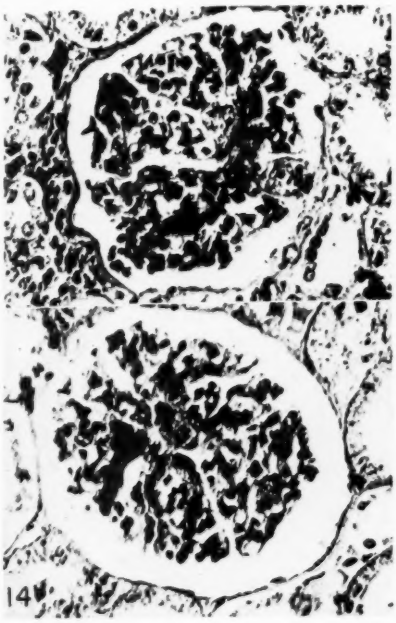
PLATE 63

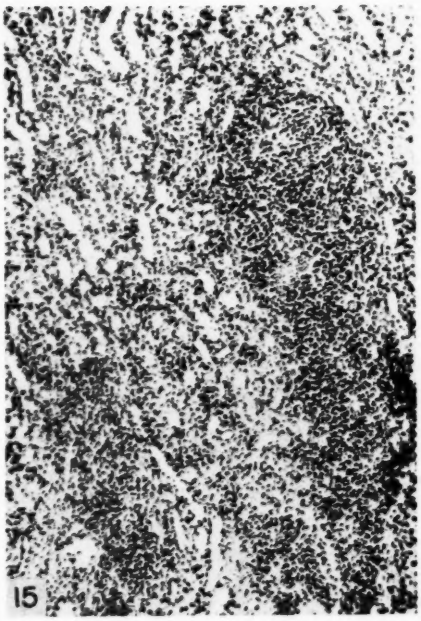
FIG. 14. Focal embolic nephritis in the dog with bacterial endocarditis. Masson's trichrome stain. × 250.

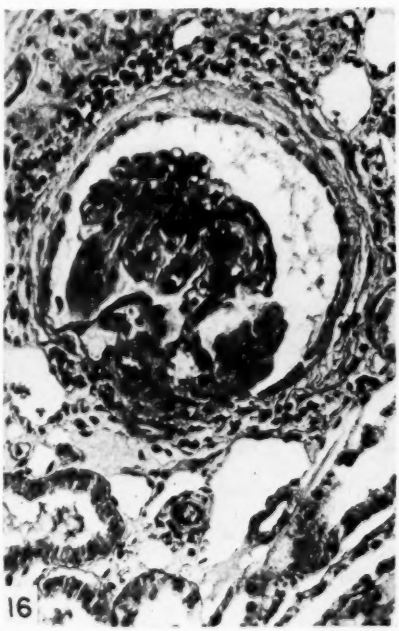
FIG. 15. Large numbers of mononuclear cells surrounding the tubules. Hematoxylin and eosin stain. × 110.

FIG. 16. Fibrous thickening of the parietal layer of Bowman's capsule. The glomerulus is surrounded by mononuclear cells. Hematoxylin and eosin stain. × 250.

FIG. 17. Destruction of renal parenchyma and replacement by fibrous tissue. There are large numbers of mononuclear cells and heavy calcium deposits. A sclerotic arteriole is seen in the lower part of the field. Hematoxylin and eosin stain. × 110.


14


15


16

17

Morehead and Little

Blood Vessels of Apparently Healthy Dogs

UNIV. OF MICH.

MYOBLASTOMA

(GRANULAR CELL MYOBLASTOMA OR MYOBLASTIC MYOMA)*

A. R. CRANE, M.D., and R. G. TREMBLAY, M.D.

(From the Pathological Laboratories, Norfolk General Hospital, Norfolk, Va., and St. John's Hospital, Brooklyn, N.Y.)

Tumors presumably composed of immature skeletal muscle elements and variously described as myoblastic myoma, myoblastoma, granular cell myoblastoma, rhabdomyoma, rhabdyome granulo-cellulaire, etc., are now recognized as a definite pathologic entity. Credit for the first clear description of these tumors under the name "myoblastic myoma" is given to Abrikossoff [5] who reported 5 cases in 1926. However, in 1854, Weber [1] reported a case of hypertrophy of the tongue in a male, 21 years old, who developed new growths after excision of the portion protruding beyond the teeth. These new growths presented microscopic features identical with the tumors now known as myoblastomas.

Other earlier reports of identical lesions were made by Heurtaux [2] (1881), Pendl [3] (1897) and Moschcowitz [4] (1922). The case reported by Heurtaux was that of a small nodule occurring on the dorsum of the tongue in a female, 19 years old, and was interpreted by him as granular-protein degeneration of muscle fibers. Pendl designated his neoplasm, occurring on the tongue of a male, 8 weeks of age, as a rhabdomyoma and explained it on the basis of misplaced embryonic tissue. Moschcowitz labelled his tumor, occurring on the dorsum of the tongue of a female, 30 years old, as a "xanthoma," describing it as a myogenetic form of xanthoma. Abrikossoff,[5] writing in 1926, described 5 cases (3 of the tongue and one each of the lip and gastrocnemius muscle), clearly stating their relationship to striated muscle but regarding the lesion as a degenerative process following trauma or inflammation. Later, however, in the light of cases reported by Klinge [10] and of subsequent cases of his own, he felt that some of the tumors might have developed from primitive myoblasts of the embryo.

Since Abrikossoff's report,[5] many instances of tumors of this type have been reported and we have been able to collect the details of 162 cases (Table I), including 5 of our own reported here.

REPORT OF CASES

Case 1

The patient was a newborn white female (S. J. S37-657), delivered of a 23-year-old primipara. Physical examination was negative save for the occurrence of three distinct growths on the gingival ridge of the maxilla at about the location of the right upper lateral incisor, the largest measuring 2 by 1.5 cm. The largest

* Received for publication, May 12, 1944.

growth was smooth, egg-shaped and pedunculated and partially covered by the smaller ones which were also pedunculated. All were covered by mucous membrane. There were no abnormal laboratory findings and roentgenograms of the bony skeleton were negative. The tumor was excised and there was no recurrence.

Grossly, the specimen consisted of four small pieces of tissue 0.7 cm. in diameter and one piece 2 by 1.5 cm. These were covered by smooth mucosa and presented firm, white to light brown cut surfaces. Microscopically (Fig. 1), the sections showed the tissue to be covered by a normal squamous epithelium and to be composed of large (30 to 70 μ), round, polyhedral, or spindle-shaped cells with acidophilic cytoplasm. The cytoplasm contained dense, coarse, acidophilic to neutrophilic granules giving it a granular appearance. The nuclei were small, round, central and vesicular, though many pyknotic forms were also present. No irregular, lobulated, or atypical tumor nuclei were noted nor were mitoses seen. No lipoidal material was identified with fat stains. The stroma was loose and edematous in some portions, but for the most part the cells were closely packed with scant supporting stroma.

Case 2

The patient was a newborn colored female (S. J. S43-643), born of a 27-year-old woman who had one previous normal pregnancy with the child living and well. The physical examination was negative save for the occurrence of a smooth red tumor covered by gingival membrane and located at the gingival margin of the mandible just to the left of the midline. The tumor was excised and the child discharged. She was returned 3 days later with a history of feeding poorly and slight bleeding from the gum. At this time the physical examination showed marked listlessness and dehydration with depression of the fontanelles. The wound in the mouth was well healed. The heart and lungs were negative. The abdomen was distended and an enlarged liver could be palpated. The extremities were negative. The child was in extremis and died 4 hours after admission.

Grossly, the tumor consisted of a nodule of tissue 1.5 by 0.8 by 0.8 cm. and covered over one surface by a smooth membrane. The cut surfaces were soft, golden-yellow and fatty in appearance. Microscopically, the tissues was composed of cords and masses of large, round, polyhedral, or spindle-shaped cells separated by a loose, edematous, fibroblastic tissue (Fig. 2). The cells had a coarsely granular, eosinophilic cytoplasm and showed small, central, round, vesicular nuclei (Fig. 3). Some of the cells were elongated, cylindrical or teardrop in shape, the longer ones showing one or two points of narrowing and containing two or three nuclei in different portions of the cell. With phosphotungstic acid and hematoxylin stain many cells showed fine, fibrillary, cross and longitudinal, purple fibrils which did not traverse the entire length of the cells (Fig. 4). The cells also showed a purple to blue, granular appearance of portions of the cell membrane.

Mallory's aniline blue stains showed similar fibrils and granular structures staining red and in a linear arrangement, and very coarse granulations staining dark purple (Fig. 3). The connective tissue extended around individual cells as fine strands of collagen but at some points the cells were in direct apposition. No mitoses were noted. Special stains for fat were negative.

The autopsy showed no evidence of tumor at the site of the original lesion. The only positive findings were in the liver and kidneys. The liver was enlarged (150 gm.) and presented a smooth yellow surface and a firm yellow cut surface. Microscopically, the liver cells were swollen and distorted by large, clear, fat droplets. The portal zones showed an apparent increase in bile ducts as seen in biliary cirrhosis. The kidneys were grossly normal but showed many fat droplets in the convoluted tubules with a special stain, but no other abnormalities. The anatomic diagnoses were: imbalance of fat metabolism with fatty metamorphosis of the liver and lipoid nephrosis, biliary cirrhosis of the liver, cerebral edema.

Case 3

The patient was a Negro, 26 years of age (S. V. 18566). A tumor the size of a walnut was removed from the anal region. Further clinical details are not available.

Grossly, the tumor measured 4 by 3 by 2.2 cm., was covered by skin and showed a red-gray, firm cut surface. Microscopically, the tumor was composed of strands and masses of cells which were polygonal to rounded and often elongated. The cells were acidophilic, finely granular, and occurred singly and in solid masses. The stroma was dense and fibrous. Special stains for fat were negative. The overlying squamous epithelium showed some downgrowth of the rete pegs and melanotic pigment had been mobilized, being present in macrophages in the papillary layer.

Case 4

A white female, 29 years old (S. V. 10324), who had injured her left knee 8 years previously, noted a mass the size of a pea on the inner side of the thigh just above the knee. This grew to the size of a small walnut. There were a few long hairs over the nodule. There was some pain in the region of the knee but the lesion proper was not tender. Physical examination was negative save for the local lesion, which was attached to the skin but not to the bone or muscle. The lesion was excised locally and there has been no recurrence.

Grossly, the tumor was 1.5 cm. in diameter, sharply circumscribed, and presented a firm, gray-white cut surface. Microscopically, the tumor was composed of groups and cords of polyhedral to spindle-shaped eosinophilic cells separated by a dense fibrous tissue. The cells

TABLE I

Cases of Myoblastoma Reported in the Literature

Year	Author	Sex	Age	Duration	Location	Size	Remarks
1854	Weber[1]	M	21	Years	Tongue	Diffuse overgrowth	
1881	Heurtaux[2]	F	19	2 years	Dorsum of tongue	Small nodule	
1897	Pendl[3]	M	8 wks.	Life	Dorsum of tongue	Pigeon's egg	
1922	Moschcowitz[4]	M	30	Months	Dorsum of tongue	1 cm.	
1926	Abrikossoff[5]	M		Months	Dorsum of tongue	0.8 x 0.5 cm.	
		F	30	1 year	Gastrocnemius	6 x 10 cm.	Local recurrence, 3 mos.
		M	25	Months	Tongue	1 cm.	
		M		Weeks	Upper lip	Cherry	Central ulceration
		M			Dorsum of tongue	0.5 cm.	
1926	Keynes[6]	M	26	9 months	Dorsum of tongue	0.5 cm.	Proliferation of epithelium
1926	Rütz[7]	M	5 mos.	Life	Dorsum of tongue	Plum	
1927	Dewey[8]	F	42	1 year	Dorsum of tongue	1.5 cm.	
1927	Diss[9]	M	59	3 months	Tongue	Pea	
1928	Klinge[10]				Tongue	Pea	
					Tongue	Pea	
					Tongue	Pea	
					Skin (2 cases)		
1928	Roffo[11]	M	16	Weeks	Tongue		
1929	Jaulin and Grandclaude[12]	F	42	Months	Dorsum of tongue	Pea	
1929	Volkmann[13]	F	Nb*	Life	Maxilla	Plum	"Congenital epulis"
1930	Diss[14]	M	38	Months	Dorsum of tongue	0.7 cm.	
1931	Abrikossoff[15]	M	37	2 months	Dorsum of tongue	0.5 cm.	
		M	21	1 year	Skin	1.5 cm.	
		M	24	Years	Vocal cord	Pea	
		F	55	2 months	Breast	2 x 1.5 cm.	
		F	29		Esophagus	Pea	
		M	36	1 year	Mandible	Fist	Giant myoblasts
		M	55	10 years	Mandible	Hen's egg	Giant myoblasts
1931	Bolanos and Despaigne[16]	F	12	Months	Tongue	Pigeon's egg	Hyperkeratosis of epithelium
1931	Ceelen[17]	M	50	3–4 years	Tongue	0.75 cm.	Proliferation of epithelium
		F	8 da.	Life	Maxilla	Pea	"Congenital epulis"; mitoses of tumor cells
		F		1½ years	Skin, pelvis	Pigeon's egg	
			Nb*	Life	Maxilla	Bean	"Congenital epulis"
1931	Dawydow[18]	M	30	1 year	Vocal cord	Pea	Proliferation of epithelium, resembling early carcinoma

		M	30	1 year	Vocal cord	Pea	Proliferation of epithelium, resembling early carcinoma
1931	Derman and Golbert[19]	M	40	1 year	Tongue	0.8 x 1.3 cm.	Mitoses
		F	32		Tongue	Small nodule	Mitoses
		M	30	1 year	Vocal cord	Lentil	Proliferation of epithelium
1932	Meyer[20]		Nb*	Life	Maxilla	4.0 x 6.0 x 9.0 cm.	No recurrence, 2 years
		F	23		Breast	Walnut	No recurrence, 6 months
		M	69	3 weeks	Tongue	0.4 x 0.8 cm.	No recurrence, 3 months
1932	Schirmer[21]	M	36	7 weeks	Tongue	Hazelnut	Overlying squamous cell carcinoma
1933	Glasunow[22]	M	32	4 months	Dorsum of tongue	1.0 cm.	
1933	Roffo[23]	F	31	3 years	Dorsum of tongue	Small nodule	
1934	Geschelin[24]	F	40	Months	Vocal cord	Pea	
1934	Geschickter[25]				5 of muscles of extremities 3 of breast 3 of subcutis in sacral region Ear Lip Tongue		Details not given
1934	Kleinfeld[26]	M	50	2 years	Vocal cord	1 cm.	Epithelial proliferation
1934	Klemperer[27]	M	58	20 years	Skin, groin	Pecan	Well, 8 months
		F	23	1 year	Skin, pubis		Well, 2 years
		M	50	2 years	Vocal cord	1 cm.	Same case reported by Kleinfeld
		M	40	6 months	Tongue	Pea	Epithelial proliferation
		M	41	Years	Skin, thigh	Cherry stone	Epithelial proliferation
		F	42	12 years	Calf	7.5 cm.	Well, 1 year
1935	Gander[28]	F	52	6 months	Dorsum of tongue	Pea	
1935	Kernan and Cracovaner[29]	F	21	3 months	Vocal cord	Pea	No recurrence, 10 months
1935	Martinez[30]	F	50	18 years	Dorsum of tongue	1.5 cm.	
1935	Morpurgo[31]	F	65	Months	Tongue	Small nodule	Mitoses: carcinoma of overlying epithelium
					Tongue Muscle, extremity	Small nodule	
1935	Seiffert[32]	M	47	Months	Dorsum of tongue	Pea	
1936	Bobbio[33]				Tongue		
1936	Lattes[34]				Tongue		
1936	Parreira and Nunes de Almeida[35]	F	16		Tongue	Small nodule	
		F	51		Tongue	Small nodule	

Table continued on following pages.

* Nb indicates newborn infant, age not specified.

TABLE I—*continued*

Year	Author	Sex	Age	Duration	Location	Size	Remarks
1937	Bang[36]	M	63	2 years	Dorsum of tongue	Small nodule	Epithelial proliferation; well, 5 years
1937	Cappell and Montgomery[37]	M	61	9 days	Bladder	2.5 cm.	
		F	27	Months	Urethral meatus	Nutmeg	No recurrence, 6 years
		M	52		Spermatic cord	6.0 x 4.5 cm.	
1937	Gray and Gruenfeld[38]	F	30	5 months	Tongue	Hazelnut	No recurrence, 9 years
		M	35	6 months	Tongue	0.6 cm.	No recurrence, 6 years
		F	55	Weeks	Skin, breast	1.5 cm.	
		F	30	1½ years	Tongue	Pea	No recurrence, 3 years
		F			Breast	10.0 cm.	
1937	Szathmary[39]	F	32	Months	Skin, perineum		
1938	Ducuing, Ducuing and Bassal[40]	M	34	20 months	Dorsum of tongue	Pea	Epithelial proliferation; well, 6 months
		M	52	2 months	Dorsum of tongue	Pea	Epithelial proliferation; well, 1 year
		M	22	2 months	Dorsum of tongue	1.5 cm.	Epithelial proliferation; well, 1 year
		M	44	6 months	Dorsum of tongue		Well, 6 months
1938	Frenckner[41]	M	25	1 week	Arytenoid	3.5 x 0.75 cm.	Cross and longitudinal striations; no recurrence in 2½ years
		F	28	1 year	Trachea	Hazelnut	No recurrence, 3 years; also reported by Bergstand
		M	41	3 years	Lower lip	1.0 x 1.0 x 1.3 cm.	
		F	22	3 years	Tongue	Small nodule	
		M	48	1 month	Tongue	Pea	
1938	von Bahr[42]	F	49	Years	Lacrimal sac	0.4 cm.	
1939	Civatte and Ducourtioux[43]	F	36		Tongue	Small nodule	
1939	Dustin[44]				Tongue		
1939	Eickhoff[45]	M	25	6 months	Tongue		Overlying squamous cell carcinoma
1939	Fräsdorf[46]	M	68		Pharynx	2 small nodules	
1939	Geschickter and Maseritz[47]	F	62		Muscle, arm		Well, 14 months
		F	45	1 year	Muscle, pectoral		Well, 14 months
		M			Muscle, arm		Well, 4 years
		M			Muscle, thigh		No recurrence
					Subcutis, sacrum		No details given
					Subcutis, sacrum		No details given
1939	Kramer[48]	F	15	14 weeks	Bronchus		
1939	Leroux and Delarue[49]	M	37	3 months	Tongue	1.5 cm.	Epithelial proliferation
		M	45	3 months	Tongue	Pea	
		M	Nb*	Life	Maxilla	1.0 x 0.5 cm.	
1941	Grayzel and Friedman[50]	F	45	Months	Axilla	Walnut	
1941	Kratochvil[51]				Maxilla		
1941	Lascano–Gonzalez[52]	M	26	15 days	Tongue	1.2 x 0.8 x 0.7 cm.	
			Nb*	Life	Maxilla	1.2 x 0.9 x 0.8 cm.	

		M	26	15 days	Tongue	1.2 x 0.8 x 0.7 cm.	
			Nb*	Life	Maxilla	1.2 x 0.9 x 0.8 cm.	
1941	Tamis and Kowles[63]	F	39	3 years	Labia	Small lump	
1941	Thoma[64]	F	30	1½ years	Tongue	1.5 cm.	
1942	Iglauer[55]	F	41	1½ years	Arytenoid	Hazelnut	
1942	Monteiro[56]				Tongue		
1942	Tuta and Schmidt[57]	F	40		Breast	1.0 x 0.7 x 0.5 cm.	
		F	69	3 years	Skin, iliac	2.5 x 2.0 x 1.0 cm.	
		F	7		Skin, sternum	0.8 cm.	
		M	47	3 years	Skin, shoulder	3.0 x 2.5 x 2.0 cm.	
1943	Altmann[58]	M	60	20 years	External auditory canal		No recurrence, 18 months
		F	77	5 years	External auditory canal		
		F	27	5 years	External auditory canal		
1943	Horn and Stout[59]	F	60	20 years	Ext. auditory canal		Also reported by Altmann
		F	77	5 years	External auditory canal		Also reported by Altmann
					5 of tongue		
					1 of lip		
					2 of maxilla or mandible		
					1 of floor of mouth		
					1 of trachea		
					3 of skin		
					10 of subcutis		
					1 of muscle, extremity		
					2 of breast		
					1 of vocal cord		Also reported by Kernan and Cracovaner
1894	Massin[60]	F	Nb*	Life	Maxilla†		2 nodules; large granular cells
1929	Kleine[61]	F	Nb*	Life	Maxilla†		No recurrence, 1½ years
1932	Jorge, Nudelman and Gringauz[62]	F	Nb*	Life	Mandible†		Large granular cells; well, 3 years
		F	Nb*	Life	Mandible†		Large granular cells
		F	Nb*	Life	Mandible†		Large granular cells

* Nb indicates newborn infant, age not specified.

† Recorded as congenital epulis of newborn.

We believe that the case reported by Imperatori [66] as a rhabdomyoma belongs in that group, although others have included it with the myoblastomas. Cases reported by Lino,[54] v. Meyenburg,[65] Giardino,[66] Cooper [67] and Bailey [68] were examined and discarded. Cases reported by Kraneis,[69] Lepage,[70] Jorge and Brachetto Brian [71] and La Manna [72] were not available to us.

were finely granular and had small vesicular nuclei. Mitoses were not noted. A special stain for fat was negative.

Case 5

The patient was a white male, 47 years old (S. V. 13400), who complained of a hard spot on his tongue of 2 months' duration. There was no history of injury. The physical examination was negative save for the occurrence of a firm, bean-like nodule at the lower right margin of the tongue. It was not ulcerated. The lesion was excised locally without recurrence.

The gross findings are not known. Microscopically, the lesion was composed of masses of large, granular, polyhedral to ribbon-like cells intermingled with skeletal muscle. The nuclei were small and vesicular. No mitoses were seen. At some points adult skeletal muscle cells ended in bulbous granular swellings similar in appearance to the cells composing the major portion of the lesion.

Morphologic Features

Gross Characteristics. The tumors reported as myoblastomas average 1 to 2 cm. in diameter, the largest one being 10 cm. in diameter, and the majority are sharply circumscribed or truly encapsulated. They may, however, be poorly demarcated and irregularly penetrate the underlying tissues. Those located under the mucous membrane are usually covered by a smooth, intact epithelium, although some reported cases have shown ulceration of the epithelium and others hyperkeratotic, or even malignant proliferation of the epithelium. They may be nodular or lobulated but usually present a solitary, distinct, round mass. Frequently they are pedunculated, polypoid, or sessile. On section they have firm, gray to yellowish-gray or tan surfaces.

Microscopic Characteristics. The tumors are composed of large polyhedral cells, the majority being irregularly rounded, but transitional forms having a tear-drop, spindle, or cylindrical shape may also be present. The rounded forms vary from 20 to 60 μ in diameter, while the spindle or cylindrical forms are much longer, measuring up to 300 μ in our own cases. Under low magnification the cells appear to occur singly or in groups, but with higher magnifications and special stains for connective tissue, such as Mallory's aniline blue, Masson's trichrome or van Gieson's, delicate strands of connective tissue are found to penetrate among and surround the majority of the cells, but small clusters of cells do occur in direct apposition to one another. In some portions the collagen framework is abundant and edematous in polypoid lesions.

The most characteristic feature is the cytoplasm of the cells which is abundant, acidophilic and granular. The granules vary from the fine acidophilic to large, coarse forms that are neutrophilic. It must be em-

phasized that the cells are granular and *not* vacuolated and that special stains for lipoid are negative. Some authors [72,73] have suggested that the cells may contain glycogen and mucin-like substances but adequate studies with proper fixation have not been done to warrant any statement in regard to these substances. Longitudinal and cross striations have been demonstrated but not constantly, and Klemperer[27] has described an arrangement of the granules in rows resembling fibrils. With immediate proper fixation, and staining (Zenker's and Mallory's phosphotungstic-acid-hematoxylin and aniline blue stains) our own cases show clear-cut, fine, longitudinal and cross fibrils.

The nuclei are small (7 to 10 μ) and vesicular, but pyknotic forms may be numerous. They are round to oval and centrally located. In our own cases some of the longer, cylindrical forms contain 2 or 3 nuclei. Mitoses are usually absent although Ceelen,[17] Derman and Golbert [19] and Morpurgo [31] reported their occurrence.

An additional associated finding is the proliferation of the overlying epithelium first mentioned by Keynes,[6] noted by many others and emphasized by Klemperer.[27] Some cases of hyperkeratosis of the epithelium have been reported and cases reported by Schirmer,[21] Morpurgo [31] and by Eickhoff [45] showed squamous cell carcinomas of the overlying epithelium, while Leroux and Delarue [49] reported a case associated with a basal cell carcinoma.

Differential Diagnosis. These tumors are most likely to be interpreted as xanthomas, the first two cases reported here having been so diagnosed originally and case 1 so reported in the literature.[74] The total absence of fat differentiates them from the true xanthomas. Geschickter [25] reported 2 cases that were misinterpreted as chordomas. So-called congenital epulis of the newborn, as described by Massin,[60] Kleine [61] and Jorge, Nudelman and Gringauz,[62] presents features identical with the myoblastomas and we believe that they should be so regarded. The tumors are also to be differentiated from rhabdomyomas and rhabdomyosarcomas which are composed of adult skeletal muscle elements.

Clinical Features

Incidence as to Age and Sex. These tumors occur at all ages and with equal frequency among males and females, as shown in Table II; they are not more common in males as previously supposed. Forty-seven per cent of the cases have occurred in persons under the age of 50 years, with the greatest incidence occurring in the third, fourth and fifth decades. Lesions of the alveolar processes occur chiefly in children. All of the maxilla were in newborn infants, but two of three tumors of the mandible occurred in the third and fifth decades.

Distribution. The location of the tumors in various portions of the

body is indicated also in Table II. The tongue is by far the most common site, 38 per cent of reported cases occurring in this location and the vast majority being located on the dorsal surface. Skin, subcutaneous tissue, muscle, alveolar processes, respiratory tract, breast, ear, and lip, in decreasing order of frequency, account for the majority of the other cases. The skin lesions are reported from the pelvic region, extremities and breast. Isolated instances have also occurred in the anus, bladder,

TABLE II

Location, Age and Sex Distribution of 162 Cases of Myoblastoma

Age in decades	1–9		10–19		20–29		30–39		40–49		50–59		60–69		70–79		Totals			
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Age and sex not given	Final
Alveolar process																			2	2
Mandible		4					1				1						2	4		6
Maxilla	1	6															1	6	4	11
Breast						1						1						2	6	8
Ear						1								1		1		3		3
Larynx and vocal cord					2	1	2			2	1						5	3		8
Lip									1								1		3	4
Muscle								1		2				1				4	9	13
Skin		1			1	1		1	3		1	1		1			5	5	7	17
Subcutis						1				1								2	15	17
Tongue	2		1	3	6	1	8	6	7	2	3	3	2	1			29	16	16	61
Trachea and bronchi				1		1												2	1	3
Other*					1 A	2 EU		1 V		1 L	1 S		2 BP				4	4	1 M	9
Totals	3	11	1	4	10	9	11	9	11	8	7	5	4	4	0	1	47	51	64	162

* A = anus, B = bladder, E = upper esophagus, L = lacrimal sac, M = floor of mouth, P = pharynx, S = spermatic cord, U = urethra, V = vulva.

This table includes our own cases and those collected from the literature, as presented in Table I.

esophagus, lacrimal gland, floor of the mouth, pharynx, spermatic cord, urethra and vulva. Stout [75] has recently encountered 2 examples in the uterus. While more than one nodule may occur at one site, there are no instances of tumors occurring at more than one place in one patient.

Lesions. The tumors occur as firm, small (1 to 2 cm.) nodules that may be polypoid or sessile. They may increase slowly in size. The overlying epithelium is usually intact but may be ulcerated. They are nontender.

Course. The lesion is a benign one and local excision will effect a complete cure. However, if incompletely removed they may recur locally, as reported by Horn and Stout; [50] and Geschickter [25] reported a tumor starting in the sacral region which showed metastases to the groin. The metastases were treated with radium and the patient was

well 20 months later. Frankly malignant tumors, as in the cases of v. Meyenburg,[65] should be more properly classified with the rhabdomyosarcomas. The longest reported duration prior to medical attention was 20 years.

Discussion

The new cases recorded here are identical with the previously recorded cases of myoblastoma, being composed of large cells with a coarsely granular cytoplasm and small vesicular nuclei. Of particular significance are the various cellular forms as noted in case 2 in which cylindrical cells were found, and the demonstration of definite longitudinal and cross fibrils. Figure 4 is a composite camera lucida drawing showing the various types of elongated cells and the fibrils.

There has been much contention as to the nature of these tumors. One group believes them to be true neoplasms composed of immature, proliferating skeletal muscle cells (myoblasts), while others regard the lesions as degenerative, interpreting the cells as muscle fibers undergoing necrobiotic changes. Abrikossoff,[5] in his first paper, regarded the tumors as resulting from a degenerative process following trauma or inflammation in striated muscle, but in reporting his second series of cases,[15] in 1931, he stated that some of the tumors might originate from primitive myoblasts representing embryologic rests (Cohnheim), as suggested by Klinge [10] and even earlier by Pendl [3] and Rütz.[7]

The concept that these lesions are composed of proliferating myoblasts has been based upon the morphologic resemblance of the cells to immature skeletal muscle fibers as described by Godlewski.[76] He described myoblasts as showing granules occurring in rows and later the formation of homogeneous fibrils. Wolbach,[77] in 1928, in studies of rhabdomyomas of the heart and breast, again noted the linear arrangement of the cytoplasmic granules and believed that the myofibrils were formed by the connection of these granules which, he stated, develop from the multiplication and dispersion of centrioles and centriole clusters. Klemperer [27] described a similar arrangement of granules in one of his cases, and this is also evident in our case 2. The occurrence of the cross striations has also supported the myogenous nature of these tumors, and Geschickter [25,47] as well as others has placed much emphasis on the occurrence of longitudinal fibrils. The development of the tumors in sites apart from muscular tissue is presumably due to the close embryologic association of the skin and muscular anlage. According to Maximow and Bloom,[78] the lower part of the medial layer of each primitive segment of the embryo forms the sclerotome while the lateral layer of the segment produces the skin plate. The remaining portion of the medial layer, the myotome, represents the primordium

of the dorsal and ventral musculature of the body. The occurrence of various skin tumors has been explained on the basis of imperfect separation, muscular elements being carried in the skin plate. Pendl [3] was the first to suggest the development of these myomas from misplaced embryonal tissues and subsequently the hypothesis of their development from embryologic rests has been embraced by many authors.

The original concept of Abrikossoff [5] that these lesions are a degenerative process of skeletal muscle has been championed particularly by Gray and Gruenfeld,[38] who demonstrated granular cells in degenerating skeletal muscle cells in a nonspecific ulceration of the tongue. They object to the designation of these tumors as myoblastomas on their presumed resemblance to the myoblasts described by Godlewski [76] and cite the works of Zechel,[79] Franz [80] and Marcus [81] on myogenesis, which make no reference to granular forms. Furthermore they were unable to demonstrate granular cells in embryos of man and laboratory animals. The transitional forms so frequently seen in the tongue they regard as skeletal muscle cells undergoing necrobiotic changes. Heurtaux,[2] Abrikossoff,[5] Roffo,[11] Gander,[28] Martinez [30] and Civatte and Ducourtioux [43] were all impressed by the probable degenerative nature of these lesions. Gray and Gruenfeld, and Gander suggest that all of the tumors described as myoblastomas are not histogenetically identical, regarding the lingual lesions as due to necrobiosis, while leaving the exact nature of the various other tumors open to question. This applies to our own cases, as the lesion of the tongue shows intermediate forms suggestive of muscle degeneration while this was not evident in any of the other lesions. The fact that the myoblastomas are stationary or slow-growing while striated muscle tumors are usually highly malignant is also used as an argument against the neoplastic nature of the myoblastomas.

At the present time conclusions as to the histogenesis of these lesions are not warranted. It may well be that some represent true neoplasms while others, as those of the tongue, are the result of a local degeneration of skeletal muscle.

Summary

From the literature 157 cases of myoblastoma have been collected, to which are added 5 of our own, occurring in the maxilla, mandible, tongue, subcutis and anus.

The tumors occur with equal frequency in both sexes, in all age groups, and are widely distributed over the body.

Histologically identical tumors occur in sites containing skeletal muscle and also at points normally showing no skeletal muscle elements. The most common site is the tongue and mouth, but lesions occur in other portions of the body as well.

Myoblastomas are benign tumors and complete local excision will effect a complete cure.

Definite conclusions as to the histogenesis of the myoblastomas are not warranted at the present time, the evidence being equally well founded for the myoblastic nature of some tumors and the degenerative nature of others.

We are indebted to Dr. Arnold F. Strauss of the Hospital of St. Vincent de Paul of Norfolk, Virginia, for contributing to this paper cases 3, 4 and 5 which were originally diagnosed by him, and to Harriet Cross Crane for the camera lucida drawings.

REFERENCES

1. Weber, C. O. Anatomische Untersuchung einer hypertrophischen Zunge nebst Bemerkungen über die Neubildung quergestreifter Muskelfasern. *Virchows Arch. f. path. Anat.*, 1854, **7**, 115–125.
2. Heurtaux. Tumeur de la langue (dégénérescence granulo-protéique des fibres musculaires). *J. de méd. de l'ouest, Nantes*, 1881, **15**, 305.
3. Pendl, F. Über ein congenitales Rhabdomyom der Zunge. *Ztschr. f. Heilk.*, 1897, **18**, 457–468.
4. Moschcowitz, E. Xanthoma (xanthelasma) of the tongue. *Proc. New York Path. Soc.*, 1922, **22**, 135–141.
5. Abrikossoff, A. Über Myome, ausgehend von der quergestreiften willkürlichen Muskulatur. *Virchows Arch. f. path. Anat.*, 1926, **260**, 215–233.
6. Keynes, G. Rhabdomyoma of tongue. *Brit. J. Surg.*, 1926, **13**, 570–572.
7. Rütz, A. Angeborenes Rhabdomyom der Zunge. *Med. Klin.*, 1926, **22**, 1072.
8. Dewey, K. W. Rhabdomyoma of the tongue. *Arch. Path.*, 1927, **3**, 645–657.
9. Diss, A. Un nouveau type de tumeur musculaire: le rhabdomyome granulo-cellulaire. *Bull. Assoc. franç. p. l'étude du cancer*, 1927, **16**, 863–866.
10. Klinge, F. Über die sogenannten unreifen, nicht quergestreiften Myoblastenmyome. *Verhandl. d. deutsch. path. Gesellsch.*, 1928, **23**, 376–382.
11. Roffo, A. H. Miolisis nodular de la lengua. *Bol. Inst. de med. exper. para el estud. y trat. del cancer*, 1928, **4**, 237–243. Also: *Prensa méd. argent.*, 1928–29, **15**, 78–81.
12. Jaulin and Grandclaude. Un cas de rhabdomyome granuleux de la langue. *Bull. Assoc. franç. p. l'étude du cancer*, 1929, **18**, 395–397.
13. Volkmann, J. Eine seltene, angeborene Oberkiefergeschwulst bei einem Neugeborenen (Myoblastenmyom). *Zentralbl. f. Chir.*, 1929, **56**, 2982–2983.
14. Diss, A. Le rhabdomyome granulo-cellulaire de la langue. *Ann. d'anat. path.*, 1930, **7**, 1071–1076.
15. Abrikossoff, A. I. Weitere Untersuchungen über Myoblastenmyome. *Virchows Arch. f. path. Anat.*, 1931, **280**, 723–740.
16. Bolaños, J. M., and Despaigne, E. Contribución al estudio de los rabdomiomas de la lengua. *Bol. Liga contra el cáncer*, 1931, **6**, 97–106.
17. Ceelen, W. Über Myoblastengeschwülste. *Virchows Arch. f. path. Anat.*, 1931, **280**, 741–748.
18. Dawydow, I. Zur Frage der unausgereiften Rhabdomyome des Kehlkopfes. *Ztschr. f. Hals-, Nasen- u. Ohrenk.*, 1931–32, **30**, 221–227.
19. Derman, G. L., and Golbert, Z. W. Über unreife, aus der quergestreiften Muskulatur hervorgehende Myome. *Virchows Arch. f. path. Anat.*, 1931, **282**, 172–180.
20. Meyer, R. Myoblastentumoren ("Myoblastenmyome" Abrikossoff). *Virchows Arch. f. path. Anat.*, 1932–33, **287**, 55–81.

21. Schirmer, R. Über ein Myoblastenmyom zusammen mit Cancroid der Zunge. *Beitr. z. path. Anat. u. z. allg. Path.*, 1932, **89**, 613–632.
22. Glasunow, M. Über unreife, begrenzt und destruierend wachsende Rhabdomyoblastome. *Frankfurt. Ztschr. f. Path.*, 1933, **45**, 328–345.
23. Roffo, A. H. Knotige Zungenmyolyse. *Ztschr. f. Krebsforsch.*, 1933, **39**, 464–470. Miolosis nodular de la lengua. *Bol. Inst. de med. exper. para el estud. y trat. del cáncer*, 1932, **9**, 489–502.
24. Geschelin, A. I. Fall von Myoblastomyom des Kehlkopfs. *Acta oto-laryng.*, 1934, **21**, 66–70.
25. Geschickter, C. F. Tumors of muscle. *Am. J. Cancer*, 1934, **22**, 378–410.
26. Kleinfeld, L. Myoblastoma of the larynx. *Arch. Otolaryng.*, 1934, **19**, 551–555.
27. Klemperer, P. Myoblastoma of the striated muscle. *Am. J. Cancer*, 1934, **20**, 324–337.
28. Gander, G. Du rhabdomyome granulocellulaire de la langue. *Bull. Assoc. franç. p. l'étude du cancer*, 1935, **24**, 56–63.
29. Kernan, J. D., and Cracovaner, A. J. Rhabdomyoma of the vocal cord. *Laryngoscope*, 1935, **45**, 891–893.
30. Martinez, E. M. Sobre una observación de rabdomioma gránulo-celular (Diss). *Arch. de med. int.*, 1935, **1**, 281–287.
31. Morpurgo, B. Mioblastomi. *Arch. per le sc. med.*, 1935, **59**, 229–252.
32. Seiffert, A. Myoblastenmyom der Zunge. *Ztschr. f. Laryng., Rhin., Otol.*, 1935, **26**, 4–6.
33. Bobbio, A. Mioblastoma ad elementi granulosi (mioblastomioma di Abrikossoff) della laringe. *Arch. per le sc. med.*, 1936, **61**, 583–589.
34. Lattes, R. Mioblastoma ad elementi granulosi della lingua. *Arch. per le sc. med.*, 1936, **61**, 590–595.
35. Parreira, H., and Nunes de Almeida, J. Dois casos de rabdomioma da lingua. *Arq. de pat.*, 1934, **6**, 582–600. Also: *Int. Abst. Surg.*, 1936, **62**, 36.
36. Bang, F. Rhabdomyome granulo-cellulaire de la langue. *Ugesk. f. laeger*, 1937, **99**, 710–714.
37. Cappell, D. F. and Montgomery, G. L. On rhabdomyoma and myoblastoma. *J. Path. & Bact.*, 1937, **44**, 517–548.
38. Gray, S. H., and Gruenfeld, G. E. Myoblastoma. *Am. J. Cancer*, 1937, **30**, 699–708.
39. Szathmary, Z. [Case of perineal myoblastoma.] *Magyar orvosi arch.*, 1937, **38**, 260–267.
40. Ducuing, J., Ducuing, L., and Bassal. Le rhabdomyome granulo-cellulaire de la langue. *Presse méd.*, 1938, **46**, 1018–1020.
41. Frenckner, P. The occurrence of so-called myoblastomas in the mouth and upper air passages. *Acta oto-laryng.*, 1938, **26**, 689–701.
42. von Bahr, G. A case of myoblastic myoma of the lacrimal sac. *Acta ophth.*, 1938, **16**, 109–115.
43. Civatte, A., and Ducourtioux, M. Un nouveau cas de rhabdomyome de la langue. *Bull. Soc. franç. de dermat. et syph.*, 1939, **46**, 1458–1462.
44. Dustin, A. P. À propos du myome myoblastique. *Acta, Union internat. contre cancer*, 1939, **4**, 684–685.
45. Eickhoff, H. Myoblastenmyom und Carcinom. *Virchows Arch. f. path. Anat.*, 1939, **304**, 432–441.
46. Fräsdorf, W. Zur dysontogenetischen Entstehung der "Myoblastenmyome." *Beitr. z. path. Anat. u. z. allg. Path.*, 1939, **102**, 24–35.
47. Geschickter, C. F., and Maseritz, I. H. Affections of muscles. *J. Bone & Joint Surg.*, 1939, **21**, 576–594.

48. Kramer, R. Myoblastoma of the bronchus. *Ann. Otol., Rhin. & Laryng.*, 1939, **48**, 1083–1086.
49. Leroux, R., and Delarue, J. Sur trois cas de tumeurs à cellules granuleuses de la cavité buccale. *Bull. Assoc. franç. p. l'étude du cancer*, 1939, **28**, 427–447.
50. Grayzel, D. M., and Friedman, H. H. Myoblastoma of the thoracic wall. *Arch. Path.*, 1941, **31**, 512–515.
51. Kratochvil, K. Beitrag zum Vorkommen der Myoblastengeschwülste. *Arch. f. klin. Chir.*, 1941, **201**, 83–88.
52. Lascano-Gonzalez, J. M. Observaciones de "miomas mioblásticos" (Abrikossoff). *Rev. Asoc. méd. argent.*, 1941, **55**, 300–304. Also: *Arch. Soc. argent. de anat. norm. y pat.*, 1939, **1**, 812–824.
53. Tamis, A. B., and Kowles, J. J. Myoblastoma of labium majus. *Am. J. Obst. & Gynec.*, 1941, **42**, 543–544.
54. Thoma, K. H. Rhabdomyoma of tongue. *Am. J. Orthodontics* (Oral Surg. Sect.), 1941, **27**, 235–244.
55. Iglauer, S. Myoblastoma of larynx. *Ann. Otol., Rhin. & Laryng.*, 1942, **51**, 1089–1093.
56. Monteiro, A. Mioblastoma da lingua. *Acta med., Rio de Janeiro*, 1942, **9**, 113–122.
57. Tuta, J. A., and Schmidt, F. R. So-called myoblastoma. *Arch. Dermat. & Syph.*, 1942, **46**, 225–233.
58. Altmann, F. Granular cell myoblastomas of external auditory meatus. *Laryngoscope*, 1943, **53**, 195–202.
59. Horn, R. C., Jr., and Stout, A. P. Granular cell myoblastoma. *Surg., Gynec. & Obst.*, 1943, **76**, 315–318.
60. Massin, W. N. Ein Fall von angebornem Epitheliom, entstanden aus dem Schmelzorgan. *Virchows Arch. f. path. Anat.*, 1894, **136**, 328–335.
61. Kleine, H. O. Die angeborenen Basalzelltumoren der Gingiva. Beitrag zur Histogenese der sogenannten Epulis congenita. *Arch. f. Gynäk.*, 1929, **138**, 297–317.
62. Jorge, J. M., Nudelman, S. I., and Gringauz, M. Tumores congénitos de la encía; Angiolinfangioma xantelomatoso del reborde gingival. *Semana méd.*, 1932, **39**, pt. 1, 1933–1936.
63. Imperatori, C. J. Rhabdomyoma of the larynx. *Laryngoscope*, 1933, **43**, 945–948.
64. Lino, G. Contributo allo studio dei tumori rari della lingua e della istogenisi del rabdomioma. *Tumori*, 1928, **14**, 373–378.
65. v. Meyenburg, H. Die quergestreifte Muskulatur. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1929, **9**, pt. 1, 468.
66. Giardino, G. Su alcune forme poco comuni di neoplasmi della lingua (angiomi, rabdomiomi). *Gior. ital. di mal. esot. et trop.*, 1934, **7**, 99–104.
67. Cooper, K. G. Plasmocytoma and rhabdomyoma of the paranasal sinuses. *Arch. Otolaryng.*, 1934, **20**, 329–339.
68. Bailey, W. H. Rhabdomyoma. *J. Oklahoma M. A.*, 1934, **27**, 408–409.
69. Kraneis, H. Zur Kenntnis der Rhabdomyome der Mundhöhle. Inaugural Dissertation, Bonn, 1928.
70. Lepage, R. Un cas de rabdomyome des régions maxillaires et sub-linguales. *J. de méd. de Paris*, 1934, **54**, 690–692.
71. Jorge, J. M., and Brachetto Brian, D. Rabdomioma retrofaríngeo. *Hosp. argent.*, 1935, **5**, 551–557.
72. Holle, G. Ueber die Deutung der sogenannten Myoblastenmyome als Speicherzellgeschwülste auf Grund einer besonderen färberischen Reaktion. *Zentralbl. f. allg. Path. u. path. Anat.*, 1941, **76**, 244–247.

73. von Albertini, A. Zur Frage der Myoblastenmyome der Zunge. *Schweiz. Ztschr. f. allg. Path. u. Bakt.*, 1938, **1**, 431–439.
74. Battaglia, J., and Curphey, T. J. Gingival tumor of newborn. *Am. J. Dis. Child.*, 1939, **57**, 1404–1407.
75. Stout, A. P. Personal communication.
76. Godlewski, E. Die Entwicklung des Skelet- und Herz-muskelgewebes der Säugethiere. *Arch. f. mikr. Anat.*, 1902, **60**, 111–156.
77. Wolbach, S. B. Centrioles and the histogenesis of the myofibril in tumors of striated-muscle origin. *Anat. Rec.*, 1927–28, **37**, 255–273.
78. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1942, ed. 4, pp. 167–171.
79. Zechel, G. Über Muskelknospen beim Menschen, ein Beitrag zur Lehr von der Differenzierung des Myotoms. *Ztsch. f. d. ges. Anat.*, 1924, **74**, Abt. 1, 593–607.
80. Franz, A. W. Das Problem der uni- oder multizellulären Entwicklung der quergestreiften Muskelfasern. *Arch. f. mikr. Anat.*, 1915–16, **87**, 364–491.
81. Marcus, H. Ueber die Struktur und die Entwicklung quergestreifter Muskelfasern, besonders bei Flügelmuskeln der Libellen. *Anat. Anz.*, 1919–20, **52**, 410–416.
82. La Manna. Über Myoblastome. *Virchows Arch. f. path. Anat.*, 1935, **294**, 663–691.

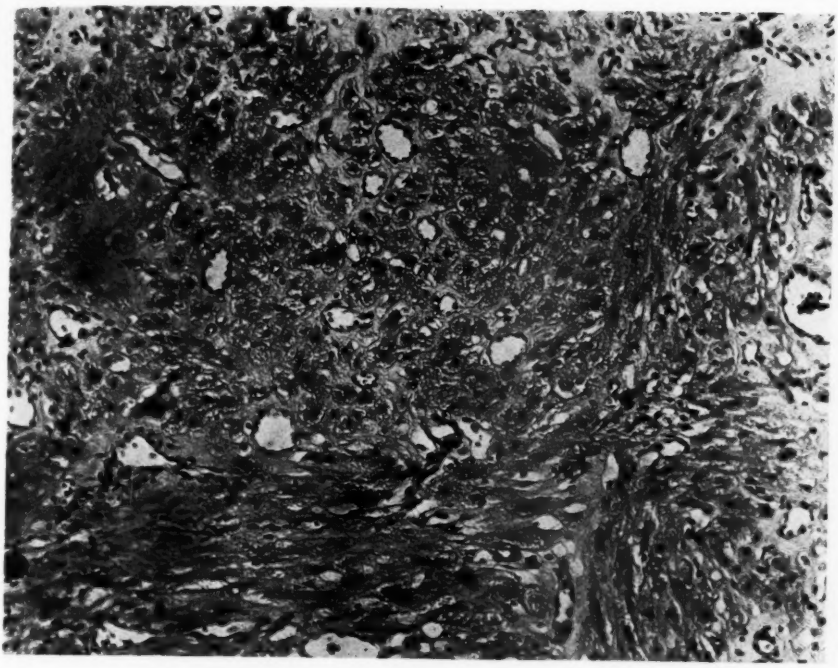
DESCRIPTION OF PLATES

PLATE 64

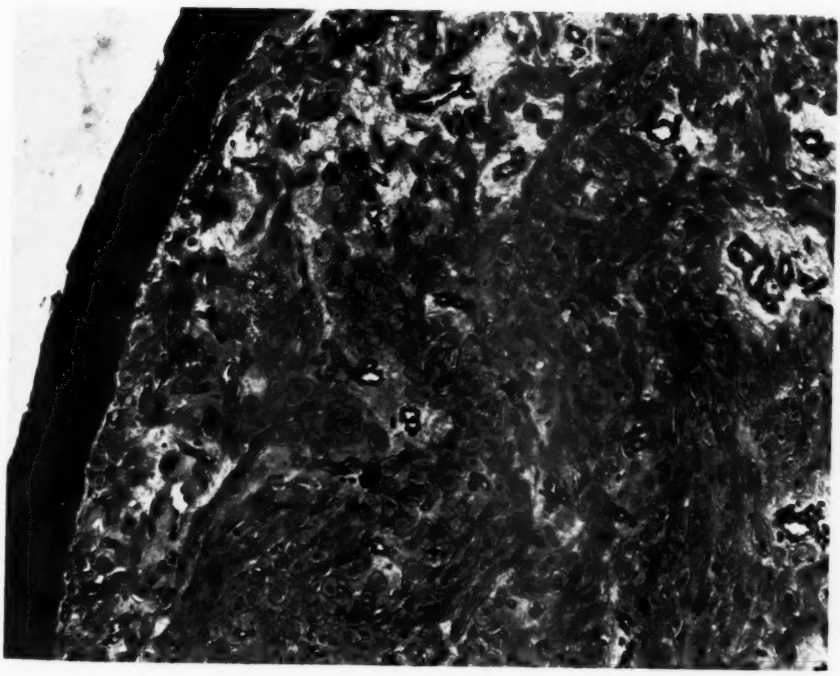
FIG. 1. Case 1. Tumor of the maxilla showing the large polyhedral to cylindrical, granular cells. Hematoxylin and eosin stain. × 150.

FIG. 2. Case 2. Tumor of the mandible showing polyhedral to cylindrical, granular cells identical with those shown in Figure 1. Some forms show more than one nucleus. Mallory's aniline blue stain. × 150.

1



2



Crane and Tremblay Myoblastoma

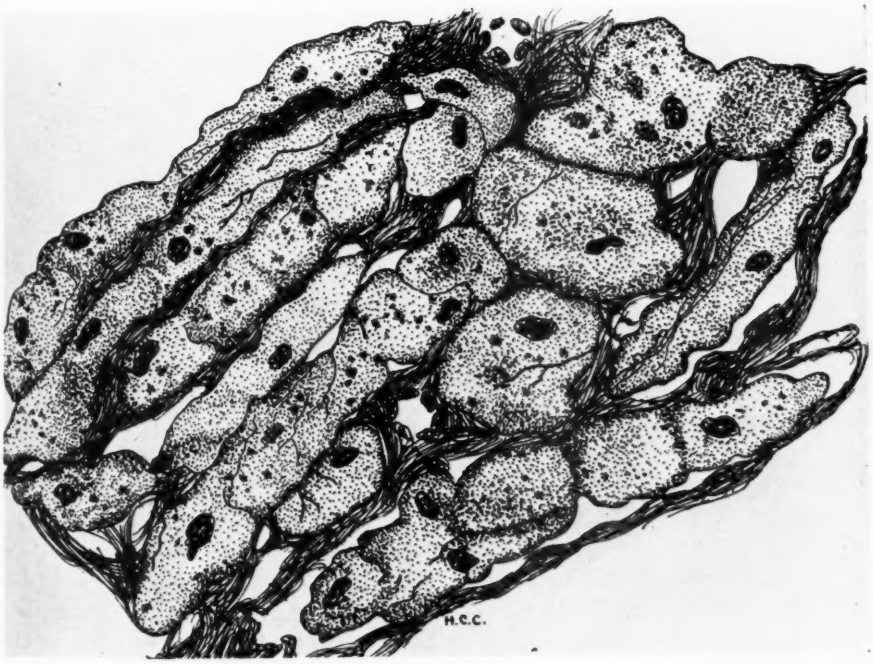
Plate 65

Fig. 3. Case 2. Same field as that shown in Figure 2 but at a higher magnification, showing the coarse granularity of the cells and the irregular fibrils traversing the cells. Mallory's aniline blue stain. × 675.

Fig. 4. A composite camera lucida drawing of case 2 showing the detail of the longer cylindrical forms with multiple nuclei and fibrils. × 700.

3

4



Crane and Tremblay Myoblastoma

VACCINAL INFECTION OF THE CHORIOALLANTOIC MEMBRANE OF THE TURTLE EMBRYO *

PAUL N. HARRIS, M.D.
(From the Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.)

The description in 1931 by Woodruff and Goodpasture [1] of infection of the chorioallantoic membrane of the chick embryo by fowl-pox virus introduced a new method of investigation of viral infections. Their technic has been applied so fruitfully to the study of other viruses that a vast literature has accrued during the past 13 years.

The experiments hitherto reported have been performed solely upon avian embryos. It seemed not unreasonable to suppose that attempts to induce viral infection of membranes of reptilian embryos might yield additional information.

METHOD

Eggs of two species of turtles, *Pseudemys troostii* and *Chrysemys picta dorsalis* were candled, and only those were used in which the embryos appeared to be in an intermediate stage of development. The shell over the back of the embryo was painted with an antiseptic solution, and after several minutes the shell was scraped away with a scalpel in a region about 2 mm. in diameter. More antiseptic solution was then applied to the exposed shell membrane, and after another pause to permit germicidal action, the membrane was punctured by a 24 gauge hypodermic needle and the suspension of virus was injected. After inoculation, the defect in the shell was sealed by collodion, the eggs were embedded in moist sand and covered with damp absorbent cotton. Eggs which were inoculated with vaccine virus were kept at 37° C., and those which were inoculated with fowl-pox virus were kept at room temperature (approximately 28° to 35° C.).

Because of turgescence of the eggs and absence of an air sac, the technical difficulties encountered were great, and it must be admitted that turtle eggs are not ideal for this type of experiment. Approximately 1500 eggs were obtained for this investigation, but only 46 were actually inoculated. Many eggs were infertile, others contained dead embryos, and many had reached such a late stage of development as to be no longer usable. Frequently, upon puncture of the shell membrane, the shell split almost explosively over half or more of the length of the egg, and even if the shell failed to split, albumin invariably exuded through the perforation in the shell membrane, as much as 0.5 to 1 cc. being lost. Aspiration of an additional 0.2 to 0.5 cc. of albumin was performed on some eggs before the virus was injected. An

* Received for publication, April 20, 1944.

attempt to obviate splitting of the shell by placing the eggs in hypertonic (10 per cent) NaCl solution for 10 to 15 minutes before treatment met with some success, but this did not prevent leakage of albumin after puncture of the shell membrane. It seems probable that this maneuver succeeded largely because it wet the shell thoroughly. Furthermore, if the eggs were allowed to become dry the shells split spontaneously.

Vaccine Virus

Twenty-one eggs were inoculated with commercial vaccine virus. Undiluted virus was used for 2 eggs, and for all others the virus was diluted with from 1 to 5 volumes of normal NaCl solution. The volume of the inocula ranged from 0.01 to 0.6 cc. Sixteen eggs were opened and examined 2 days after inoculation, 3 were opened 3 days after inoculation, and 2 were examined 5 days after inoculation. Membranes were fixed in Zenker's fluid and stained by Perdrau's [2] modification of Mann's stain.

No lesions were found in the membranes of 8 eggs. In 6 eggs there was a small hole in the chorioallantoic membrane surrounded by slight thickening, and in the remaining 7, single or multiple opaque white spots were found in the chorioallantoic membrane. Microscopic examination revealed, in the membranes from 10 eggs, proliferation and hypertrophy of ectodermal cells alone, or of both ectodermal and entodermal cells, sometimes with leukocytic infiltration of mesoderm and ectoderm. This reaction could be attributed to the presence of the virus only in eggs nos. 3 and 8, which were incubated for 5 days after inoculation, since Guarnieri bodies were not seen in membranes from any of the other eggs.

The membrane from egg no. 8 contained a lesion 2 by 4 mm. in diameter. Sections revealed thickening of both ectoderm and entoderm by cell proliferation and hypertrophy, and thickening of the mesoderm with but little cell proliferation. There was moderate leukocytic infiltration of ectoderm and mesoderm. A region of slight ectodermal ulceration was seen. Large, finely granular Guarnieri bodies were present in the cytoplasm of many ectodermal cells, and some cells contained two inclusions. Mitoses were easily found in both ectoderm and entoderm. The membrane from egg no. 3 contained multiple small lesions. Some sections were, in general, similar to those of egg no. 8, although Guarnieri bodies were less abundant. Other sections showed ectodermal ulceration and much leukocytic infiltration and fibroblastic proliferation in the mesoderm.

Six alligator eggs were inoculated with 0.05 cc. each of undiluted commercial vaccine virus after windows had been made in the shell and shell membrane. Two days later 3 embryos were dead, and the membranes of the other 3 had become infected as a result of bacterial contamination. Sections of the membranes showed

heavy leukocytic infiltration, and no Guarnieri bodies. Because of the opacity of the shell which makes candling very difficult, the absence of an air sac, and the tendency of the shells to crack on drying, these eggs are ill suited to this type of experiment.

Fowl-pox

Twenty-five turtle eggs were inoculated with from 0.05 to 0.4 cc. of fowl-pox virus suspension. This was prepared by grinding the membrane from an infected chick embryo in 1.5 cc. of 50 per cent glycerine and diluting with 3 cc. of normal NaCl solution. The eggs were examined 4 to 6 days later. At this time 7 embryos were dead and 5 were hatching. The membranes of 4 showed no gross lesions, and the membranes of 9 showed slight thickening. Sections contained no inclusions, although slight ectodermal and entodermal proliferation and leukocytic infiltration of the mesoderm were seen.

Discussion

It was our original design to observe the effects of several viruses upon the embryos of turtles, alligators, and snakes, but the experiments which had been planned could not be carried out on the intended scale because of remoteness from the source of supply, and because of the short seasonal limitation on the availability of eggs. Although several dozen eggs of different species of snakes were obtained, none could be inoculated because of infertility or death of the embryos before arrival at the laboratory. The results are presented now since work cannot be resumed here, and with the hope that others who have at hand a source of supply of reptilian, amphibian and piscine eggs will be thereby encouraged to take up the investigation and will find herein helpful ideas as to procedures.

The low incidence of vaccinal infection observed in this experiment was doubtless due to the difficulty of inoculation. The procedure was a blind one, and it was impossible to be sure that the inoculum was placed upon the chorioallantoic membrane. In fact, it is highly probable that most inoculations were made into the allantoic cavity or into the albumin sac. Creation of an artificial air sac over the chorioallantoic membrane would obviate this difficulty. However, demonstration of Guarnieri bodies in the chorioallantoic ectodermal cells of 2 eggs indicates the susceptibility of membranes of turtle embryos to vaccinal infection. In view of the susceptibility of membranes of chick embryos to infection by a wide variety of viruses, and to the susceptibility of the cornea and skin of young turtles to vaccinal infections as shown by Andervont,[3] it is to be expected that the membranes of turtle embryos would be susceptible to vaccinia.

The inclusions observed in the membranes of turtle embryos are quite similar to those which develop in the membranes of chick embryos. Differentiation of leukocytes from Guarnieri bodies is easy since

the latter are finely granular, lie within the cytoplasm of the ectodermal cells and are surrounded by a clear zone which in some cells is quite broad.

Failure of the eggs inoculated with fowl-pox virus to develop inclusions is not interpreted as indicating lack of susceptibility. However, it is stated by Findlay [4] that the tortoise and frog are resistant to fowl-pox, and it may be that membranes of turtle embryos are also resistant.

Summary

1. The chorioallantoic membrane of the turtle embryo reacts to the presence of vaccine virus by the formation of finely granular Guarnieri bodies.

2. The technic of inoculating turtle eggs and the difficulties encountered are described.

I am indebted to Dr. Clinton L. Baker, Director of the Reelfoot Lake Biological Station of the Tennessee Academy of Science, for obtaining the turtle eggs, and to Dr. E. W. Goodpasture of Vanderbilt University for an expression of opinion as to the nature of the inclusions found in the membranes.

REFERENCES

1. Woodruff, A. M., and Goodpasture, E. W. The susceptibility of the chorioallantoic membrane of chick embryos to infection with the fowl-pox virus. *Am. J. Path.*, 1931, **7**, 209–222.
2. Perdrau, J. R. Ammonium molybdate as a mordant for Mann's stain and the Weigert-Pal method. *J. Path. & Bact.*, 1939, **48**, 609–610.
3. Andervont, H. B. The susceptibility of fowls and reptiles to the vaccine virus. *Am. J. Hyg.*, 1927, **7**, 804–810.
4. Findlay, G. M. Fowl-pox. In: A System of Bacteriology in Relation to Medicine. Medical Research Council, H. M. Stationery Office, London, 1930, **7**, 261–267.

DESCRIPTION OF PLATE

Plate 66

In order to facilitate identification of Guarnieri bodies in these illustrations, the distance of the center of the inclusion from the right margin and upper margin of the prints respectively will be given in millimeters.

Fig. 1. Egg. no. 8. Six Guarnieri bodies are seen at 116 and 26, 109 and 11, 72 and 7, 54 and 7, 15 and 43, and 8 and 38. The third and fourth lie at opposite poles of the nucleus of a single ectodermal cell. Granularity of the inclusions is obvious. × 620.

Fig. 2. Egg no. 8. Twelve Guarnieri bodies are seen at 93 and 7, 93 and 17, 61 and 12, 61 and 26, 53 and 15, 49 and 9, 45 and 13, 43 and 17, 37 and 9, 16 and 10, 26 and 20, and 14 and 20. The last two lie at opposite poles of the nucleus of a single ectodermal cell. Several leukocytes with large black cytoplasmic granules are seen in the thickened ectoderm. × 620.

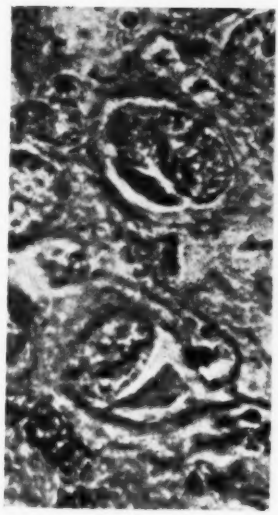
Fig. 3. Egg no. 8. Guarnieri bodies are seen at 19 and 27, and 17 and 58. × 1200.

Fig. 4. Egg no. 8. Guarnieri bodies are seen at 22 and 9, and 22 and 65. A leukocyte is seen at the right center. × 1200.

Fig. 5. Egg no. 3. Guarnieri bodies are seen in adjacent cells at 23 and 32, and 23 and 42. × 1200.

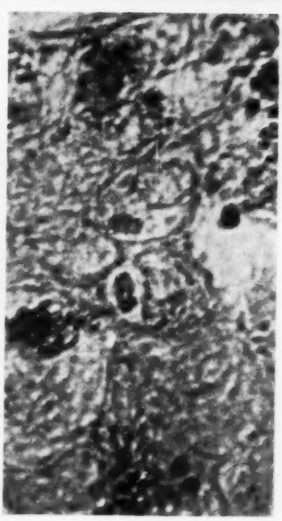
1

2



3

4



5

Harris

Vaccinal Infection of Chorioallantois of the Turtle

UNIV. OF MICH.

MULTICENTRIC BILATERAL CARCINOMA OF THE KIDNEYS *

JAMES R. LISA, M.D.
(From the Laboratory of Pathology, City Hospital, Welfare Island, Department of Hospitals, New York, N.Y.)

The case described herein is reported because it presents an unusual if not a unique variety of primary carcinoma of the kidney. Both kidneys were the site of multiple small tumors arising from the tubules, and giving rise to extensive metastases. As far as could be determined, a similar case has not been previously recorded.

The patient was a white woman, 65 years of age, with mild diabetes mellitus whose history and course suggested a malignant growth, the origin of which could not be determined clinically. Pyelonephritis and hematuria were discovered during cystoscopic examination.

Necropsy was performed 3 hours after death. The most conspicuous finding was marked lymphadenopathy of the abdominal and thoracic nodes and small nodules in the pleura, lungs, adrenals, ileum, stomach and kidneys. The nodes were enlarged, soft, gray-white and showed a few small hemorrhages. The node at the hilum of the liver was firmly adherent and locally invasive. The kidneys were enlarged: the right weighed 250 gm.; the left, 240 gm. The capsules stripped easily and the cortical surfaces were smooth. The organs were studded with small gray-white masses measuring 1 to 2 cm. in diameter. The masses in the stomach and ileum were covered with normal mucosa and in the latter organ were located in the tips of the valvulae. The anatomic diagnosis was lymphosarcoma with metastases.

The histologic study of the renal masses showed tumors in which the normal pattern was well preserved. They consisted of tubules separated by capillaries and lined by a single layer of large opaque cells which varied moderately in size. The nuclei were large and hyperchromatic, and mitoses were frequent. An occasional glomerulus was enmeshed at the periphery. In one section, a glomerulus was found which showed a layer of tumor cells lining the capsular basement membrane and a solitary tumor cell lying on the glomerular basement membrane. There was slight evidence of compression of the adjacent renal parenchyma but occasionally a tubule extended like a finger into the normal tissue. The tumors of the other organs were identical carcinomas. The final diagnosis was multiple primary carcinoma of the kidneys with metastases to the abdominal and thoracic lymphatics, pleura, lungs, adrenals, ileum and stomach.

* Received for publication, April 17, 1944.

DESCRIPTION OF PLATE

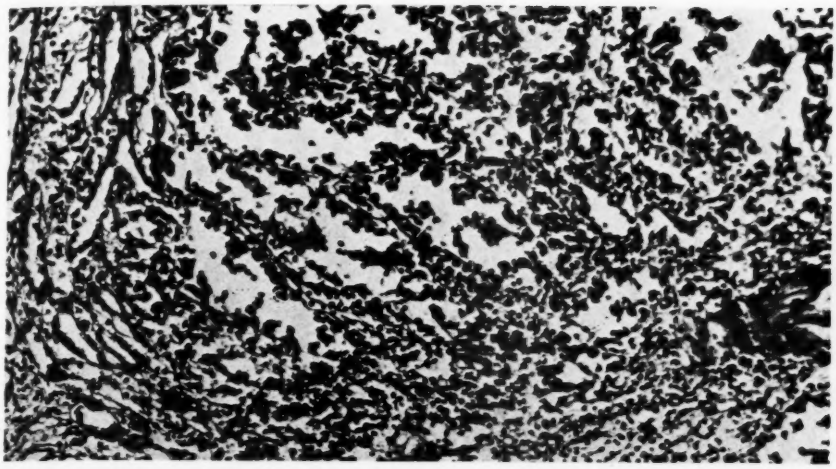
PLATE 67

FIG. 1. Photomicrograph of a renal tumor showing the tubular structure separated by a capillary network. × 90.

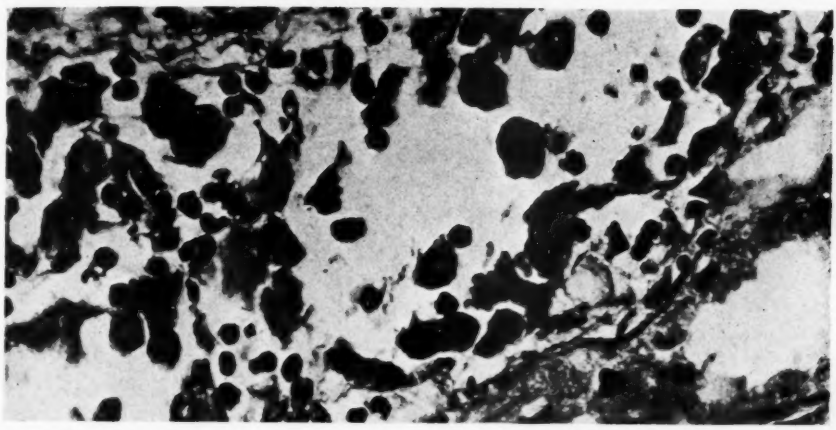
FIG. 2. Photomicrograph showing the type of tumor cell lining the tubules. × 400.

FIG. 3. Photomicrograph showing a glomerulus with malignant capsular epithelium. × 400.

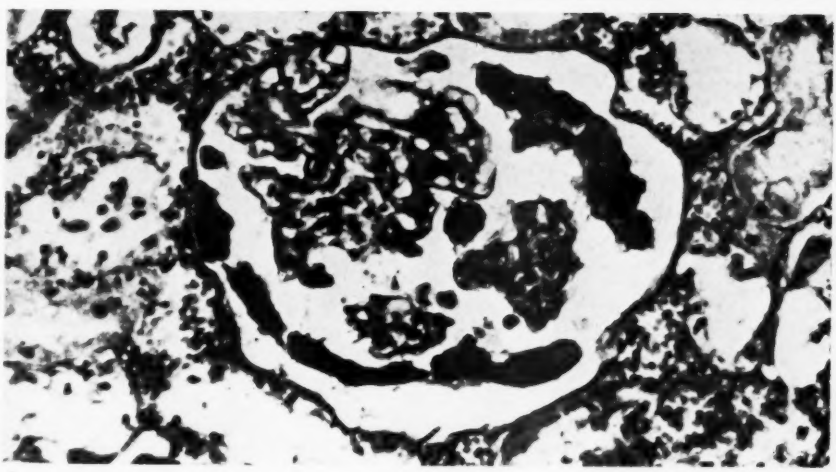
1



2



3



Lisa

Multicentric Bilateral Renal Carcinoma

UNIV. OF MICH.